

Circular RNA-delivery mediated by virus like particles

The present invention relates to a virus like particle (VLP) which comprises an artificial circular RNA molecule comprising an RNA element of interest. The present invention further relates to a method for producing a virus like particle (VLP) which comprises an artificial circular RNA molecule comprising an RNA element of interest.

BACKGROUND OF THE INVENTION

RNA is being used for therapeutic interventions via different mechanistic pathways. There are approaches to inhibit expression of genes e.g. by applications of siRNA or antisense RNA, but there exist also multiple techniques to induce gene expression by applying therapeutic active RNA as it is currently done successfully in clinical use in almost countless vaccinations against the SARS-CoV-2 virus by inducing the expression of the spike protein of SARS-CoV-2.

RNA applications have – in contrast to therapeutic treatments with DNA - the huge advantage that the attained effects are transient without risking any modification of the chromosomal DNA, thus preventing potential harmful long-term consequences. Since RNA has a rather short half-life and cannot be incorporated into the chromosomal DNA such negative consequences can be omitted completely. The drawback, however, is that RNA mediated effects take place only for several days, which seems to be sufficient at least for priming the immune response against novel viral threats. Due to the fact that RNases are highly efficient in degrading RNA molecules in rather short time, long lasting effects are rather difficult to achieve and require recurring applications of the RNA based therapy.

One way to improve the stability of therapeutically active RNA transcripts has been achieved by taking benefit of circular RNA (circRNA), a covalently closed single-stranded RNA molecule that can be generated within eukaryotic cells by a non-canonical RNA splicing mechanism called backsplicing. Initially, circRNA was thought to play a role mainly in viruses such as the hepatitis D virus or plant viroids, but meanwhile circRNA has been found in fungi, plants, insects, fish and mammals – supported by next-generation-sequencing of RNA in high-throughput approaches.

Unlike linear mRNA, circular RNAs (circRNA) is highly stable as its covalently closed ring structure protects it from exonuclease-mediated degradation. Novel possibilities for therapeutic interventions are currently investigated in detail.

A big hurdle of circRNA based therapies so far is the delivery of biological active circRNA molecules into the right cell and thus, into the right organ at sufficiently high efficacies. Especially, the delivery of circRNA *in vivo* is problematic, but also *in vitro* approaches such as the modification of T-cells for CAR-T cell therapies would benefit dramatically, if the delivery of circRNA could be improved while simultaneously keeping the cytotoxic effect at a tolerable level.

The delivery of linear single-strand RNA is made possible by lipid-nanoparticle encapsulation. The clinical utility of mRNA vaccines delivered in lipid nanoparticles has recently been highlighted by their use in SARS-CoV-2 vaccines from Moderna (mRNA-1273) and Pfizer-BioNTech (BNT162b2). Lipid nanoparticles have to fulfil several functions: (i) they have to protect the cargo against nuclease in the physiological fluids once the RNA drugs are applied, (ii) they should be modified in a way to escape the mononuclear phagocyte system (MPS) and clearance by the by renal glomerular filtration especially upon systemic applications, (iii) they should be able to enter the targets cells efficiently and (iv) they need to escape out of the endosomes once internalized. To fulfil these tasks with maximal efficiency, numerous chemical modifications have been investigated with individual pro and cons. For this reason, the current mRNA vaccines still have certain limitations due to its inherent instability and suboptimal thermostability after encapsulation for *in vivo* administration, as well as potential immunogenic side effects. Lipid-nanoparticles encapsulated with RNA are very complex systems. Disadvantages include the presence of alternative colloidal structures, the complexity of the physical state of the lipid, and the possibility of super cooled melts which cause stability problems during storage or administration. Sample dilution or water removal might significantly change the equilibria between the different colloidal species and the physical state of the lipid.

Recently, circRNA vaccines against SARS-CoV-2 and circRNAs expressing nanobodies or AEC2 decoys to neutralize the SARS-CoV- where developed (Liang Qu et al. (2021): Circular RNA Vaccines against SARS-CoV-2 and Emerging Variants. doi: <https://doi.org/10.1101/2021.03.16.435594>). These circRNA were delivered via lipid-nanoparticle encapsulation, too. The limitations of this packaging method have already been described above for linear RNAs and also apply to circular RNAs.

Thus, there is an unmet need for novel safe and efficient vector production for the transmission of genetic materials in a subject, e.g. mammal.

Viruses, however, have evolved strategies over millions of years to identify routes to enter the cytoplasm of target cells efficiently. The present inventors surprisingly found that

circRNAs can be packaged into virus like particles (VLPs). By incorporating recruiting RNA motifs into the molecule to be packaged, circRNAs such as coding or non-coding circRNAs can be selectively packaged into VLPs. The recruiting RNA motifs only become functional through circularization, whereby the molecule to be packaged is packaged exclusively in its circularized state. Surprisingly, these RNAs can also be packaged in the envelopes of DNA viruses. These VLPs are capable for *in vivo* or *in vitro* delivery of (therapeutic) circRNA molecules.

SUMMARY OF THE INVENTION

In a first aspect, the present invention relates to a (linear) polynucleotide (capable of circularization) comprising in the following order from 5' to 3':

a first backsplicing intron sequence,
a first part of a recruiting RNA motif,
a nucleotide sequence of interest,
a second part of a recruiting RNA motif, and
a second backsplicing intron sequence,

wherein the first part of the recruiting RNA motif and the second part of the recruiting RNA motif form a functional recruiting RNA motif after circularization.

In a second aspect, the present invention relates to vector comprising the polynucleotide of the first aspect.

In a third aspect, the present invention relates to an artificial circular RNA molecule comprising an RNA element of interest and a recruiting RNA motif.

In a fourth aspect, the present invention relates to a complex comprising or to a complex of

- (i) a polypeptide comprising a capsid protein of a virus or a fragment or a derivative thereof having capsid protein function and at least one RNA binding domain, and
- (ii) an artificial circular RNA molecule comprising an RNA element of interest, preferably the artificial circular RNA molecule according to the third aspect.

In a fifth aspect, the present invention relates to a virus like particle (VLP) which comprises the complex according to the fourth aspect.

In a sixth aspect, the present invention relates to a virus like particle (VLP) which comprises an artificial circular RNA molecule comprising an RNA element of interest.

In a seventh aspect, the present invention relates to a method for producing a virus like particle (VLP) which comprises an artificial circular RNA molecule comprising an RNA element of interest, preferably as defined in the fifth or sixth aspect, comprising the steps of:

- (i) providing a cell, and
- (ii) introducing
 - (iia) a polypeptide comprising a capsid protein of a virus or a fragment or a derivative thereof having capsid protein function and at least one RNA binding domain, a polynucleotide encoding the polypeptide, or a vector comprising the polynucleotide, and
 - (iib) a polynucleotide according to the first aspect, a vector according to the second aspect, an artificial circular RNA molecule comprising an RNA element of interest, or an artificial circular RNA molecule according to the third aspect

into the cell,

thereby producing the virus like particle (VLP) comprising an artificial circular RNA molecule comprising an RNA element of interest.

In an eighth aspect, the present invention relates to a method for producing a virus like particle (VLP) which comprises an artificial circular RNA molecule comprising an RNA element of interest, preferably as defined in the fifth or sixth aspect, comprising the steps of:

- (i) providing a virus like particle (VLP) which comprises a polypeptide comprising a capsid protein of a virus or a fragment or a derivative thereof having capsid protein function and at least one RNA binding domain,
 - (ii) dis-assembling the VLP,
 - (iii) adding an artificial circular RNA molecule comprising an RNA element of interest or an artificial circular RNA molecule according to the third aspect,
 - (iv) re-assembling the dis-assembled VLP,
- thereby producing the virus like particle (VLP) comprising an artificial circular RNA molecule comprising an RNA element of interest.

In a ninth aspect, the present invention relates to a virus like particle (VLP) obtainable by the method according to the seventh or eighth aspect.

In a tenth aspect, the present invention relates to a composition comprising the virus like particle of according to the fifth, sixth, or ninth aspect.

In an eleventh aspect, the present invention relates to a virus like particle according to the fifth, sixth, or ninth aspect or to a composition according to the tenth aspect for use in medicine.

In a twelfth aspect, the present invention relates to a virus like particle according to the fifth, sixth, or ninth aspect or to a composition according to the tenth aspect for use in therapy.

In a thirteenth aspect, the present invention relates to a virus like particle according to the fifth, sixth, or ninth aspect or to a composition according to the tenth aspect for use in vaccination.

This summary of the invention does not describe all features of the invention.

DETAILED DESCRIPTION OF THE INVENTION

Definitions

Before the present invention is described in detail below, it is to be understood that this invention is not limited to the particular methodology, protocols and reagents described herein as these may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention which will be limited only by the appended claims. Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art.

Preferably, the terms used herein are defined as described in “A multilingual glossary of biotechnological terms: (IUPAC Recommendations)”, Leuenberger, H.G.W, Nagel, B. and Kölbl, H. eds. (1995), Helvetica Chimica Acta, CH-4010 Basel, Switzerland).

Several documents are cited throughout the text of this specification. Each of the documents cited herein (including all patents, patent applications, scientific publications, manufacturer's specifications, instructions, GenBank Accession Number sequence submissions etc.), whether supra or infra, is hereby incorporated by reference in its entirety. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

In the following, the elements of the present invention will be described. These elements are listed with specific embodiments; however, it should be understood that they may be combined in any manner and in any number to create additional embodiments. The variously described examples and preferred embodiments should not be construed to limit the present invention to only the explicitly described embodiments. This description should be understood to support and encompass embodiments which combine the explicitly described embodiments with any number of the disclosed and/or preferred elements. Furthermore, any permutations

and combinations of all described elements in this application should be considered disclosed by the description of the present application unless the context indicates otherwise.

Throughout this specification and the claims which follow, unless the context requires otherwise, the word “comprise”, and variations such as “comprises” and “comprising”, will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integer or step. As used in this specification and the appended claims, the singular forms “a”, “an”, and “the” include plural referents, unless the content clearly dictates otherwise.

The term “consisting essentially of”, as used herein, limits the scope of a claim to the specified materials or steps and those that do not materially affect the basic and novel characteristic(s) of the claimed invention. In other words, the term “consisting essentially of”, as used herein, is generally construed to mean that the composition or formulation (a) necessarily includes the listed ingredients and (b) is open to unlisted ingredients that do not materially affect the basic and novel properties of the composition. Similarly, when “consisting essentially of” is used herein in a process claim, the claim requires that the listed steps are performed, but also may include unlisted steps that do not affect the basic and material properties of the process.

The term “about” when used in connection with a numerical value is meant to encompass numerical values within a range having a lower limit that is 5% smaller than the indicated numerical value and having an upper limit that is 5% larger than the indicated numerical value.

The terms “polypeptide” and “protein” are used interchangeably in the context of the present invention and refer to a long peptide-linked chain of amino acids.

The terms “polypeptide fragment” or “protein fragment”, as used herein, refer to a polypeptide or protein having a deletion, e.g. an amino-terminal deletion, and/or a carboxy-terminal deletion, and/or an internally deletion compared to the full-length polypeptide or protein.

The term “virus”, as used herein, refers to a molecule that replicates only inside living cells of other organisms. It may also be cultivated in cell culture. Viruses can infect all types of life forms, from animals and plants to microorganisms including bacteria and archaea. While not inside an infected cell or in the process of infecting a cell, viruses exist in the form of independent particles. These viral particles, also known as virions, consist of two or three parts: (i) the genetic material made from either DNA or RNA, long nucleotides that carry genetic information, (ii) a protein coat, called the capsid, which surrounds and protects the genetic

material, and in some cases (iii) an envelope of lipids that surrounds the protein coat when they are outside a cell. The shapes of these virus particles range from simple helical and icosahedral forms for some virus species to more complex structures for others. Thus, the term “virus”, as used herein, also encompasses viral particles.

The virus, as described herein, may be an RNA virus or a DNA virus.

Preferably,

the DNA virus is selected from the group consisting of a papovavirus, a papilloma virus, a polyoma virus, an adenovirus, and a parvovirus virus, or

the RNA virus is a retrovirus, a norovirus, or an orthomyxovirus.

More preferably,

the polyoma virus is a Simian Virus 40 (SV40), a BK virus (BKV), a JC virus (JCV), or a MPy virus (MPyV),

the parvovirus is an adeno-associated virus (AAV),

the retrovirus is a lentivirus,

the norovirus is a Norwalk virus, or

the orthomyxovirus is an influenza A or influenza B virus.

The term “enveloped virus”, as used herein, refers to a virus having a viral envelope covering its protective protein capsid. The envelopes typically are derived from portions of the host cell membranes (phospholipids and proteins), but include some (viral) glycostructures such as glycoproteins and/or glycooligopeptides. Functionally, viral envelopes help viruses to enter host cells and may help them to avoid the host immune system. (Viral) glycostructures such as glycoproteins and/or glycooligopeptides on the surface of the envelopes serve to identify and bind to receptor sites on the host's membrane. The viral envelope then fuses with the host's membrane, allowing the capsid and viral genome to enter and infect the host.

The term “virus like particle (VLP)”, as used herein, refers to a virus like structure of a virus made up of one or more capsid proteins with the ability to self-assemble, mimicking the form and size of a virus but lacking the functional genetic material so it is not capable of establishing a productive infectious cycle (that includes formation of progeny) in a host cell.

The virus like particle (VLP), as described herein, may be a virus like particle of an RNA virus or a DNA virus.

Preferably,

the virus like particle of a DNA virus is selected from the group consisting of a papovavirus like particle, a papilloma virus like particle, a polyoma virus like particle, an adenovirus like particle, and a parvovirus like particle, or

the virus like particle of an RNA virus is a retrovirus like particle, a norovirus like particle, or an orthomyxovirus like particle.

More preferably,

the polyoma virus like particle is a Simian Virus 40 (SV40) like particle, a BK virus (BKV) like particle, a JC virus (JCV) like particle, or a MPy virus (MPyV) like particle,

the parvovirus like particle is an adeno-associated virus (AAV) like particle,

the retrovirus like particle is a lentivirus like particle,

the norovirus like particle is a Norwalk virus like particle, or

the orthomyxovirus like particle is an influenza A or influenza B virus like particle.

The term “capsid”, as used herein, refers to the protein shell of a virus. It consists of several oligomeric (repeating) structural subunits made of proteins called protomers. The observable 3-dimensional morphological subunits, which may or may not correspond to individual proteins, are called capsomeres. The proteins making up the capsid of a virus are called “capsid proteins”. Alternatively, they are called “viral coat proteins (VCP)”.

The term “capsid protein”, as used herein, refers to a protein making up the capsid of a virus. Thus, the function of the capsid protein is the formation of the capsid of a virus. Specifically, capsid proteins may be induced to self-assemble into a virus like particle (VLP) when one or more genes coding for the production of the capsid proteins are being provided such that a sufficiently high concentration of capsid proteins is achieved in a given volume. The capsid proteins may have to be processed for self-assembly. Processing (such as proteolytic processing) may be achieved by co-expression of processing enzymes (such as proteases) or by insertion of artificial (novel) processing sites into the capsid protein sequence. The artificial (novel) processing sites may include target sites for capsid or viral proteases, or self-processing sites such as ribosomal skipping sites such as the F2A (or 2A-like sequences) of the foot-and-mouth disease virus (FMDV).

The term “fragment of a capsid protein having capsid protein function” refers to a fragment derived from a naturally occurring capsid protein which lacks one or more amino acids, e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acid(s), compared to the naturally occurring capsid protein and has capsid protein function. In particular, said fragment of a naturally occurring capsid protein is still able to make up/form the capsid of a virus or is still able to facilitate the make up/formation of the capsid of a virus. Generally, a fragment of an amino acid sequence contains fewer amino acids than the corresponding full-length sequence, wherein the amino acid sequence present is in the same consecutive order as in the full-length sequence. As such, a fragment does not contain internal

insertions or deletions of anything into the portion of the full-length sequence represented by the fragment.

The term “derivative of a capsid protein having capsid protein function” refers to a derivative of a naturally occurring capsid protein, wherein one or more amino acids, e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acid(s), have been substituted, deleted, and/or added compared to the naturally occurring capsid protein and has capsid protein function. In particular, said derivative of a naturally occurring capsid protein is still able to make up/form the capsid of a virus or is still able to facilitate the make up/formation of the capsid of a virus. In contrast to a fragment, a derivative may contain internal insertions or deletions within the amino acids that correspond to the full-length sequence, or may have similarity to the full-length coding sequence.

The fragments or derivatives described herein can also be designated as variants. Preferably, the capsid protein has an amino acid sequence according to SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, or an amino acid sequence having at least 80%, preferably at least 85%, more preferably at least 90%, and most preferably at least 95% or 99%, i.e. 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99%, sequence identity thereto. Alternatively, the capsid protein has an amino acid sequence encompassed by the amino acid sequence according to SEQ ID NO: 31 or an amino acid sequence having at least 80%, preferably at least 85%, more preferably at least 90%, and most preferably at least 95% or 99%, i.e. 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99%, sequence identity thereto.

A capsid protein having at least 80%, preferably at least 85%, more preferably at least 90%, and most preferably at least 95% or 99%, i.e. 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99%, sequence identity to the amino acid sequence according to SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, or SEQ ID NO: 4 can be designated as a capsid protein variant. Such a capsid protein variant is still able to make up/form the capsid of a virus or is still able to facilitate the make up/formation of the capsid of a virus. The experimental section provides, for example, sufficient information in this respect. The same applies to SEQ ID NO: 31 variants.

The capsid protein of a virus or a fragment or a derivative thereof having capsid protein function, as described herein, is connected to/fused to/associated with at least one RNA binding domain. The term “RNA binding domain (also designated as aptamer binding protein (ABP))”, as used herein, refers to a structure such as a peptide or polypeptide/protein that is capable of binding to a recruiting RNA motif (also designated as aptamer). RNA binding peptides or

polypeptides/proteins exhibit highly specific recognition of their RNA targets by recognizing their sequences and structures. Specific binding of the RNA binding peptides or polypeptides/proteins allow them to distinguish their targets and regulate a variety of cellular functions via control of the generation, maturation, and lifespan of the RNA transcript.

Specifically, the term “RNA binding domain (also designated as aptamer binding protein (ABP))”, as used herein, refers to one half of a binding pair that is connected to/fused to/associated with a capsid protein of a virus or a fragment or a derivative thereof having capsid protein function and is used to associate/bring into contact an RNA element of interest with the capsid protein of a virus or the fragment or the derivative thereof having capsid protein function via binding to the other half of the binding pair, the recruiting RNA motif (i.e. the “corresponding motif”), being part of a circular RNA (circRNA) molecule comprising the RNA element of interest.

Preferably, the at least one RNA binding domain is selected from the group consisting of a MS2 coat protein or an RNA-binding section thereof, a Ku protein or an RNA-binding section thereof, a Sm7 protein or an RNA-binding section thereof, a SfMu phage COM RNA binding protein or an RNA-binding section thereof, and a PP7 coat protein or an RNA-binding section thereof.

The capsid protein of a virus or a fragment or a derivative thereof having capsid protein function can be connected to/fused to/associated with the at least one RNA binding domain via a linker.

The term “linker”, as used herein, refers to any structure which allows to connect the capsid protein of a virus or a fragment or a derivative thereof having capsid protein function with the at least one RNA binding domain. The term “linker” includes, but is not limited to, a chemical modification, a peptide linker, a chemical linker, a covalent bond, or a non-covalent bond. Preferably, the linkage is covalent. More preferably, the linker is a flexible peptide linker having a sequence consisting primarily of stretches of Glycine and Serine residues, but can contain additional amino acids such as Threonine and Alanine to maintain flexibility, as well as polar amino acids such as Lysine and Glutamic acid to improve solubility. The capsid protein of a virus or a fragment or a derivative thereof having capsid protein function and an RNA binding domain may also be present in form of a protein fusion.

The term “circular RNA (circRNA) molecule”, as used herein, refers to a type of single-stranded RNA which, unlike linear RNA, forms a covalently closed continuous loop. Specifically, in circular RNA, the 5' and 3' ends normally present in an RNA molecule have been joined together. Circular RNA (circRNA) molecules can be generated through non-

sequential back-splicing in which a downstream splice donor is linked to an upstream splice acceptor. Because circular RNA does not have 5' and 3' ends, it is resistant to exonuclease-mediated degradation and is more stable than most linear RNA in cells.

In nature, the biogenesis of circRNAs is mainly regulated by three different mechanisms: intron pairing-driven circularization, RNA-binding proteins (RBPs)-mediated circularization, and lariat-driven circularization. CircRNAs can sequester and absorb miRNAs to regulate the function of miRNAs. CircRNAs also interact with proteins and, thus, regulate their cellular localization and activity. CircRNAs promote the transcription of their parental genes by interacting with RNA polymerase II (Pol II) or U1 small nuclear ribonucleoprotein (snRNP). CircRNAs play an important role in gene regulation by competing with canonical splicing of pre-mRNAs. Intriguingly, some circRNAs are capable of encoding proteins.

The circular RNA (circRNA) molecule, as described herein, can be part of/comprised in a virus like particle (VLP). In particular, the circular RNA (circRNA) molecule, as described herein, can be packaged into a virus like particle (VLP). It is specifically an artificial circular RNA (circRNA) molecule.

The circular RNA (circRNA) molecule, as described herein, specifically comprises an RNA element of interest and/or a recruiting RNA motif. The recruiting RNA motif binds/is capable of binding to the at least one RNA binding domain that is connected to/fused to/associated with the capsid protein of a virus or a fragment or a derivative thereof having capsid protein function.

The terms “recruit”, “recruiting” or “recruitment”, as used herein, refer to attracting an element to another structure, e.g. using RNA-peptide or RNA-polypeptide/protein interactions. In the present case, the RNA element of interest is recruited via interaction of the recruiting RNA motif with the RNA binding domain to the capsid protein of a virus and the circular RNA (circRNA) molecule comprising the RNA element of interest can, thus, be encapsidated/encapsulated within the capsid of a virus like particle (VLP), as described herein.

The term “recruiting RNA motif (also designated as aptamer)”, as used herein, refers to an element that exhibits affinity for a given ligand or target with high selectivity and specificity. At the molecular level, the recruiting RNA motif/aptamer-ligand or recruiting RNA motif/aptamer-target interaction is particularly mediated through non-covalent forces such as electrostatic interactions, hydrophobic interactions, pi-pi orbital stacking, and/or hydrogen bonding interactions.

Especially, the term “recruiting RNA motif (also designated as aptamer)”, as used herein, refers to a short single-stranded RNA oligonucleotide (e.g. 25 to 70 bases) that can bind a specific ligand or target via its 3D structure.

Specifically, the term “recruiting RNA motif (also designated as aptamer)”, as used herein, refers to one half of a binding pair that is used to recruit an RNA element of interest to which the recruiting motif is bound to a capsid protein which is connected to/fused to/associated with the other half of the binding pair, the RNA binding domain (i.e. the “corresponding motif”). The recruiting RNA motif binds the RNA binding domain and, in this way, recruits the RNA element of interest to the capsid protein of a virus or a fragment or a derivative thereof having capsid protein function. In other words, in this way, the recruiting RNA motif leads to an association between the RNA element of interest and the capsid protein of a virus or a fragment or a derivative thereof having capsid protein function which allows the encapsidation/encapsulation of the circular RNA (circRNA) molecule comprising the RNA element of interest in the capsid protein structure of a virus like particle (VLP).

Preferably, the recruiting RNA motif is selected from the group consisting of a MS2 phage operator stem-loop, a telomerase Ku binding motif, a telomerase Sm7 binding motif, a SfMu phage COM stem-loop, and a PP7 phage operator stem-loop.

The RNA binding domain and the recruiting RNA motif can be a pair selected from the above groups. More preferably,

- (i) the at least one RNA binding domain is a MS2 coat protein or an RNA-binding section thereof and the recruiting RNA motif is a MS2 phage operator stem-loop,
- (ii) the at least one RNA binding domain is a Ku protein or an RNA-binding section thereof and the recruiting RNA motif is a telomerase Ku binding motif,
- (iii) the at least one RNA binding domain is a Sm7 protein or an RNA-binding section thereof and the recruiting RNA motif is a telomerase Sm7 binding motif,
- (iv) the at least one RNA binding domain is a SfMu phage COM RNA binding protein or an RNA-binding section thereof and the recruiting RNA motif is a SfMu phage COM stem-loop, and/or
- (v) the at least one RNA binding domain is a PP7 coat protein or an RNA-binding section thereof and the recruiting RNA motif is PP7 phage operator stem-loop.

For example, the coat protein of the RNA bacteriophage MS2 usually binds a specific MS2 phage operator stem-loop structure in viral RNA and the coat protein of the RNA bacteriophage SfMu usually binds a specific SfMu phage operator stem-loop structure in viral RNA to accomplish encapsidation of the genome and translational repression of replicase synthesis.

Further, the Ku protein usually binds TLC1, the RNA component of telomerase. When Ku's ability to bind TLC1 is disrupted, DNA repair via telomere healing is reduced.

Furthermore, in *Saccharomyces cerevisiae*, the telomerase RNA, TLC1, is usually bound by the Sm7 protein complex, which is required for stabilization of the predominant, non-polyadenylated (poly(A)⁻) TLC1 isoform.

The present inventors have used the above molecules for their RNA delivery approach.

The term “RNA element of interest”, as used herein, refers to an RNA sequence. The RNA element of interest has a specific function or is of a specific purpose, and is, therefore, of interest. The function of the RNA element of interest is, for example, the inhibition of the expression of genes, e.g. by application of antisense RNA. Alternatively, therapeutic active RNA may be applied to induce gene expression, e.g. to elicit an immune response. In one prominent example, the expression of the spike protein of SARS-CoV-2 is induced to elicit an immune response against the SARS-CoV-2 virus.

A problem of RNA based therapies so far is the delivery of biological active RNA molecules into the right cell and, thus, into the right organ at sufficiently high efficacies. Especially, the delivery of RNA *in vivo* is problematic, but also *in vitro* approaches would benefit dramatically, if the delivery of RNA could be improved while simultaneously keeping the cytotoxic effect at a tolerable level. Due to the fact that RNases are highly efficient in degrading RNA molecules in rather short time, long lasting effects are rather difficult to achieve and require recurring applications of the RNA based therapy. Delivery mechanism which prevent the degradation of biological active RNA molecules would be highly desirable.

The purpose of the present invention is the encapsidation/encapsulation of an RNA element of interest in a virus like particle (VLP), as described herein, in particular in the capsid protein structure of a virus like particle (VLP), as described herein. In this way, the RNA element of interest is safely packaged and can efficiently be delivered into target cells, e.g. via viral routes. As the RNA element of interest is part of a circular RNA (circRNA) molecule, which is a highly stable and covalently closed ring structure, it is further protected from exonuclease-mediated degradation.

Preferably, the RNA element of interest is selected from the group consisting of coding or non-coding RNA. More preferably, the RNA element of interest is selected from the group consisting of a shRNA, miRNA, protein encoding RNA, pre-miRNA, piRNA, RNA complementary to other non-coding RNA, and a ribozyme. Thus, the RNA element of interest may encode, in form of a protein encoding RNA, a product of interest. A product of interest may be a protein of interest. The RNA of interest may also be a non-coding RNA, e.g. a miRNA. Non-coding RNAs are frequently used to silence a corresponding target gene.

The term “heterologous RNA element of interest”, as used herein, refers to an RNA element that is either derived from another natural source, e.g. another organism, or is taken out of its natural context, e.g. fused, attached, or coupled to another molecule, or is not normally found in nature.

In particular, the term “heterologous RNA element of interest”, as used herein, refers to an RNA element that is not normally found intimately associated with the virus/virus like particle (VLP) in nature.

In a preferred embodiment, the RNA element of interest, as described herein, is a heterologous RNA element of interest.

As the capsid protein of a virus or the fragment or the derivative thereof having capsid protein function is fused to/associated with at least one RNA binding domain and as the at least one RNA binding domain binds to/interacts with the recruiting RNA motif, the RNA element of interest is brought in contact with the capsid protein and, thus, the circular RNA (circRNA) molecule comprising the RNA element of interest is encapsidated/encapsulated in the capsid structure of the virus like particle, as described herein.

The term “binding” according to the invention preferably relates to a specific binding. “Specific binding” means that a compound (e.g. an RNA binding domain, also designated as aptamer binding protein (ABP)) binds stronger to a target (e.g. a recruiting RNA motif, also designated as aptamer) for which it is specific compared to the binding to another target. A compound binds stronger to a first target compared to a second target, if it binds to the first target with a dissociation constant (K_d) which is lower than the dissociation constant for the second target. Preferably the dissociation constant (K_d) for the target to which the compound binds specifically is more than 10-fold, preferably more than 20-fold, more preferably more than 50-fold, even more preferably more than 100-fold, 200-fold, 500-fold or 1000-fold lower than the dissociation constant (K_d) for the target to which the compound does not bind specifically.

The term “ K_d ” (usually measured in “mol/L”, sometimes abbreviated as “M”) is intended to refer to the dissociation equilibrium constant of the particular interaction between a compound (e.g. a compound of the invention) and a target molecule.

Methods for determining binding affinities of compounds, i.e. for determining the dissociation constant K_d , are known to a person of ordinary skill in the art and can be selected for instance from the following methods known in the art: Surface Plasmon Resonance (SPR) based technology, Bio-layer interferometry (BLI), enzyme-linked immunosorbent assay (ELISA), flow cytometry, isothermal titration calorimetry (ITC), analytical ultracentrifugation,

radioimmunoassay (RIA or IRMA) and enhanced chemiluminescence (ECL). Typically, the dissociation constant K_d is determined at 20°C, 25°C, 30°C, or 37°C.

The circular RNA molecule comprising an RNA element of interest and/or a recruiting RNA motif, as described herein, may be produced from a linear polynucleotide which is capable of circularization.

The term “linear polynucleotide capable of circularization” (specifically in the context of covalent circularization), as used herein, refers to a DNA or RNA polynucleotide that is capable of forming a circular structure in which the 5' and 3' termini, in particular the 5' and 3' termini of one of its exons, are covalently linked with each other.

Such a circular structure can be achieved by backsplicing such as protein-assisted backsplicing or autocatalytic backsplicing. For backsplicing, it is required that the linear polynucleotide comprises 5' and 3' backsplicing intron sequences. A circular polynucleotide occurs from the linear polynucleotide, in particular from an exon of the linear polynucleotide, by backsplicing when the 5' backsplicing intron sequence is joined to the upstream 3' backsplicing intron sequence.

Thus, in order to be capable of circularization, the linear polynucleotide, as described herein, specifically comprising in the following order from 5' to 3':

- a first backsplicing intron sequence,
- a first part of a recruiting RNA motif,
- a nucleotide sequence of interest,
- a second part of a recruiting RNA motif, and
- a second backsplicing intron sequence.

The linear polynucleotide capable of circularization, as described herein, can be a DNA or RNA polynucleotide.

The backsplicing intron sequences are preferably introns containing splice sites along with short inverted repeats, such as Alu elements, to allow protein-assisted circularization of exons. More preferably, the backsplicing intron sequences are from the human ZKSCAN1 genes. In human, exons 2 and 3 of ZKSCAN1 are spliced together to form a covalently linked 668-nt circular RNA termed circZKSCAN1 via protein-assisted backsplicing. The ZKSCAN1 intron has been re-arranged so that after transcription, a heterologous single internal exon is rendered circular by protein-assisted backsplicing reaction. Even more preferably, the first backsplicing intron sequence has a nucleotide sequence according to SEQ ID NO: 34, or is a nucleotide sequence having at least 80%, preferably at least 85%, more preferably at least 90%, and most preferably at least 95% or 99%, i.e. 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98,

or 99%, sequence identity thereto and/or the second backsplicing intron sequence has a nucleotide sequence according to SEQ ID NO: 35, or is a nucleotide sequence having at least 80%, preferably at least 85%, more preferably at least 90%, and most preferably at least 95% or 99%, i.e. 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99%, sequence identity thereto.

Alternatively, the autocatalytic backsplicing intron sequences are preferably derived from group I or group II introns. More preferably, the autocatalytic backsplicing intron sequences are derived from group I intron T4 from bacteriophage T4 td gene. The intron has been split and re-arranged so that after transcription, a single internal exon is rendered circular by the group I splicing reaction. Even more preferably, the first autocatalytic backsplicing intron sequence has a nucleotide sequence according to SEQ ID NO: 43, or is a nucleotide sequence having at least 80%, preferably at least 85%, more preferably at least 90%, and most preferably at least 95% or 99%, i.e. 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99%, sequence identity thereto and/or the second backsplicing intron sequence has a nucleotide sequence according to SEQ ID NO: 44, or is a nucleotide sequence having at least 80%, preferably at least 85%, more preferably at least 90%, and most preferably at least 95% or 99%, i.e. 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99%, sequence identity thereto.

The term “protein-assisted backsplicing”, as used herein, refers to backsplicing regulated by splicing factors. circRNAs are generated from splicing of pre-mRNA in reversed orders across exons. As a variation from linear splicing, protein-assisted backsplicing requires spliceosome assembly. Two flanking backsplicing intron sequences are required to generate circular RNA.

The term “autocatalytic backsplicing”, as used herein, refers to backsplicing in absence of splicing factors. Preferably, circRNAs are generated from linear RNAs comprising self-splicing group I or group II introns. Group I and group II introns are two types of RNA enzymes, ribozymes, that catalyze their own splicing by different mechanisms. The flanking self-splicing group I and group II introns included in the linear RNA molecule may be obtained or derived from any organism, such as, for example, bacteria, bacteriophages, and eukaryotic organisms. The group I intron of phage T4 thymidylate synthase (td) gene is well characterized to circularize RNA. When the td intron order is permuted flanking any exon sequence, the exon is circularized via autocatalytic transesterification reactions.

The term “circular RNA (circRNA) molecule”, as used herein, also refers to a type of single-stranded RNA which, unlike cellular linear RNA, is artificially designed to self-anneal

into a stable non-covalent ring even in the absence of one or more RNA binding proteins such as Cap-binding proteins or poly-A-binding proteins.

The term “linear polynucleotide capable of circularization” (specifically in the context of non-covalent circularization), as used herein, refers to a DNA or RNA (or mixed DNA and RNA) polynucleotide that is capable of forming a circular structure in which the 5' and 3' termini associate with high stability in a non-covalent fashion. For example, such a noncovalent structure is formed if RNA termini together can intertwine into highly stable pseudoknots, G-quadruplexes, or base triplets similar to what is observed in the RNA component (TR) of the telomerase ribonucleoprotein complex (consisting of TERT, TR and cofactors) or the 3' end of the MALAT1 transcript. The difference to the naturally occurring pseudoknots, G-quadruplexes or base triplets being that both termini cooperate to form such a structure (whereas in naturally occurring RNAs a contiguous internal stretch, or a terminus alone folds onto itself independent of the opposite terminus). Non-covalent structures can also be formed when the two termini are designed to fold into aptameres or chelating shapes that bind a common (linking) entity such as a free nucleotide or other small molecule.

The circularizing structure in the context of the non-covalent RNA circles has the function to prevent exposure of the 5' terminus of uncapped or decapped synthetic or natural RNA (normally with the chemical structure of a 5'-monophosphate, 5'-diphosphate, or 5'-triphosphate) and at the same time to prevent exposure of the 3' end with or without a poly-A tail. Thus, the definition of having a stably non-covalently closed circle is that such a circularised RNA is not susceptible to either 5' or 3' exonucleases whereas linear polynucleotide in the same preparation is digested.

Furthermore, the circularizing structure in the context of the non-covalent RNA circles can serve as a recruiting RNA motif (also designated as aptamer). Whereas the 5' or 3' terminus by itself is not bound by the RNA binding domain the circularized structure can be recognized and recruited into a nucleotide/protein interaction. The recruitment can then be further utilized for encapsidation as a virus like particle (VLP). The nucleotide binding motif for the recruitment of the non-covalently closed circular RNA into a VLP can, for example, be obtained from proteins that are known to bind RNA triple-helices. Such protein domains can be found, for example, in the yeast ribosome-binding protein Stm1p27 or splicing factors PSF, P54nrb, or U2AF6529. The nucleotide binding motif for the recruitment of a circular RNA with a G quadruplex closure can be found, for example, in the proteins AVEN, CNBP, DDX21, DDX3X, DHX36, FMRP, nucleolin, or G3BP1.

The term “nucleotide sequence of interest”, as used herein, refers to a DNA or an RNA sequence. The DNA sequence can encode an RNA element or a protein. The RNA element or protein encoded by the DNA sequence has a specific function and is, therefore, of interest. In case the nucleotide sequence of interest is an RNA sequence, the RNA sequence itself can exert a specific function as RNA element or can encode a protein. The function of the RNA element is, for example, the inhibition of the expression of genes, e.g. by application of antisense RNA. Alternatively, therapeutic active RNA may be applied to induce gene expression, e.g. to elicit an immune response. In one prominent example, the expression of the spike protein of SARS-CoV-2 is induced to elicit an immune response against the SARS-CoV-2 virus.

Preferably, the RNA element of interest is selected from the group consisting of coding or non-coding RNA. More preferably, the RNA element of interest is selected from the group consisting of a shRNA, miRNA, protein coding RNA, pre-miRNA, piRNA, RNA complementary to other non-coding RNA, and a ribozyme. Thus, the RNA element of interest may encode a product of interest. A product of interest may be a polypeptide or protein of interest. The RNA element of interest may also be a non-coding RNA, e.g. a miRNA or RNA complementary to miRNA. Non-coding RNAs are frequently used to silence a corresponding target gene or to silence or improve expression of a group of genes.

In the linear polynucleotide capable of circularization, as described herein, the recruiting RNA motif is splitted into two parts, “a first part of the recruiting RNA motif” and “a second part of the recruiting motif”, in a way that the parts no longer have a significant recruiting function on their own. For example, the recruiting motif can be splitted as follows.

	second part of the recruiting motif	first part of the recruiting RNA motif
MS2	gcgca	catgaggatcacccatgtgc (SEQ ID NO: 45)
MS2	gcgcac	atgaggatcacccatgtgc (SEQ ID NO: 46)
MS2	gcgcaca	tgaggatcacccatgtgc (SEQ ID NO: 47)
MS2	gcgcacat	taggatcacccatgtgc (SEQ ID NO: 48)
MS2	gcgcacatg	aggatcacccatgtgc (SEQ ID NO: 49)
MS2	gcgcacatga (SEQ ID NO: 56)	ggatcacccatgtgc (SEQ ID NO: 50)
MS2	gcgcacatgag (SEQ ID NO: 57)	gatcacccatgtgc (SEQ ID NO: 51)
MS2	gcgcacatgagg (SEQ ID NO: 58)	atcacccatgtgc (SEQ ID NO: 52)
MS2	gcgcacatgagga (SEQ ID NO: 59)	tcacccatgtgc (SEQ ID NO: 53)
MS2	gcgcacatgaggat (SEQ ID NO: 60)	cacccatgtgc (SEQ ID NO: 54)
MS2	gcgcacatgaggatc (SEQ ID NO: 61)	acccatgtgc (SEQ ID NO: 55)

MS2	gcgcacatgaggatca (SEQ ID NO: 62)	cccatgtgc
MS2	gcgcacatgaggatcac (SEQ ID NO: 63)	ccatgtgc
MS2	gcgcacatgaggatcacc (SEQ ID NO: 64)	catgtgc
KU	ttctgtcgtac (SEQ ID NO: 107)	ttatagatcgctacgttatttcaattttgaaaatctgagtcctggga gtgcgga (SEQ ID NO: 65)
KU	ttctgtcgtact (SEQ ID NO: 108)	tatagatcgctacgttatttcaattttgaaaatctgagtcctggga gtgcgga (SEQ ID NO: 66)
KU	ttctgtcgtactt (SEQ ID NO: 109)	atagatcgctacgttatttcaattttgaaaatctgagtcctgggag tgcgga (SEQ ID NO: 67)
KU	ttctgtcgtactta (SEQ ID NO: 110)	tagatcgctacgttatttcaattttgaaaatctgagtcctgggagt gcgga (SEQ ID NO: 68)
KU	ttctgtcgtacttat (SEQ ID NO: 111)	agatcgctacgttatttcaattttgaaaatctgagtcctgggagt cgga (SEQ ID NO: 69)
KU	ttctgtcgtacttata (SEQ ID NO: 112)	gatcgctacgttatttcaattttgaaaatctgagtcctgggagtgc gga (SEQ ID NO: 70)
KU	ttctgtcgtacttatag (SEQ ID NO: 113)	atcgctacgttatttcaattttgaaaatctgagtcctgggagtgcg ga (SEQ ID NO: 71)
KU	ttctgtcgtacttataga (SEQ ID NO: 114)	tcgctacgttatttcaattttgaaaatctgagtcctgggagtgcgg a (SEQ ID NO: 72)
KU	ttctgtcgtacttatagat (SEQ ID NO: 115)	cgctacgttatttcaattttgaaaatctgagtcctgggagtgcgg a (SEQ ID NO: 73)
KU	ttctgtcgtacttatagatc (SEQ ID NO: 116)	gctacgttatttcaattttgaaaatctgagtcctgggagtgcgga (SEQ ID NO: 74)
KU	ttctgtcgtacttatagatcg (SEQ ID NO: 117)	ctacgttatttcaattttgaaaatctgagtcctgggagtgcgga (SEQ ID NO: 75)
KU	ttctgtcgtacttatagatcgc (SEQ ID NO: 118)	tacgttatttcaattttgaaaatctgagtcctgggagtgcgga (SEQ ID NO: 76)
KU	ttctgtcgtacttatagatcgct (SEQ ID NO: 119)	acgttatttcaattttgaaaatctgagtcctgggagtgcgga (SEQ ID NO: 77)
KU	ttctgtcgtacttatagatcgcta (SEQ ID NO: 120)	cggtatttcaattttgaaaatctgagtcctgggagtgcgga (SEQ ID NO: 78)

KU	ttctgtcgtacttatagatcgctac (SEQ ID NO: 121)	gttatttcaattttgaaaatctgagtcctgggagtgcgga (SEQ ID NO: 79)
KU	ttctgtcgtacttatagatcgctacg (SEQ ID NO: 122)	ttatttcaattttgaaaatctgagtcctgggagtgcgga (SEQ ID NO: 80)
KU	ttctgtcgtacttatagatcgctacgt (SEQ ID NO: 123)	tatttcaattttgaaaatctgagtcctgggagtgcgga (SEQ ID NO: 81)
KU	ttctgtcgtacttatagatcgctacgtt (SEQ ID NO: 124)	atttcaattttgaaaatctgagtcctgggagtgcgga (SEQ ID NO: 82)
KU	ttctgtcgtacttatagatcgctacgtta (SEQ ID NO: 125)	tttcaattttgaaaatctgagtcctgggagtgcgga (SEQ ID NO: 83)
KU	ttctgtcgtacttatagatcgctacgttat (SEQ ID NO: 126)	ttcaattttgaaaatctgagtcctgggagtgcgga (SEQ ID NO: 84)
KU	ttctgtcgtacttatagatcgctacgttatt (SEQ ID NO: 127)	tcaattttgaaaatctgagtcctgggagtgcgga (SEQ ID NO: 85)
KU	ttctgtcgtacttatagatcgctacgttattt (SEQ ID NO: 128)	caattttgaaaatctgagtcctgggagtgcgga (SEQ ID NO: 86)
KU	ttctgtcgtacttatagatcgctacgttatttc (SEQ ID NO: 129)	aattttgaaaatctgagtcctgggagtgcgga (SEQ ID NO: 87)
KU	ttctgtcgtacttatagatcgctacgttatttca (SEQ ID NO: 130)	attttgaaaatctgagtcctgggagtgcgga (SEQ ID NO: 88)
KU	ttctgtcgtacttatagatcgctacgttatttcaa (SEQ ID NO: 131)	ttttgaaaatctgagtcctgggagtgcgga (SEQ ID NO: 89)
KU	ttctgtcgtacttatagatcgctacgttatttcaat (SEQ ID NO: 132)	tttgaaaatctgagtcctgggagtgcgga (SEQ ID NO: 90)
KU	ttctgtcgtacttatagatcgctacgttatttcaatt (SEQ ID NO: 133)	ttgaaaatctgagtcctgggagtgcgga (SEQ ID NO: 91)
KU	ttctgtcgtacttatagatcgctacgttatttcaattt (SEQ ID NO: 134)	tgaaaatctgagtcctgggagtgcgga (SEQ ID NO: 92)
KU	ttctgtcgtacttatagatcgctacgttatttcaatttt (SEQ ID NO: 135)	gaaaatctgagtcctgggagtgcgga (SEQ ID NO: 93)
KU	ttctgtcgtacttatagatcgctacgttatttcaattttg (SEQ ID NO: 136)	aaaatctgagtcctgggagtgcgga (SEQ ID NO: 94)
KU	ttctgtcgtacttatagatcgctacgttatttcaattttga (SEQ ID NO: 137)	aaatctgagtcctgggagtgcgga (SEQ ID NO: 95)

KU	ttctgtcgtacttatagatcgctacgttattcaa tttgaa (SEQ ID NO: 138)	aatctgagtcctgggagtgcgga (SEQ ID NO: 96)
KU	ttctgtcgtacttatagatcgctacgttattcaa tttgaaa (SEQ ID NO: 139)	atctgagtcctgggagtgcgga (SEQ ID NO: 97)
KU	ttctgtcgtacttatagatcgctacgttattcaa tttgaaaa (SEQ ID NO: 140)	tctgagtcctgggagtgcgga (SEQ ID NO: 98)
KU	ttctgtcgtacttatagatcgctacgttattcaa tttgaaaat (SEQ ID NO: 141)	ctgagtcctgggagtgcgga (SEQ ID NO: 99)
KU	ttctgtcgtacttatagatcgctacgttattcaa tttgaaaatc (SEQ ID NO: 142)	tgagtcctgggagtgcgga (SEQ ID NO: 100)
KU	ttctgtcgtacttatagatcgctacgttattcaa tttgaaaatct (SEQ ID NO: 143)	gagtcctgggagtgcgga (SEQ ID NO: 101)
KU	ttctgtcgtacttatagatcgctacgttattcaa tttgaaaatctg (SEQ ID NO: 144)	agtctctgggagtgcgga (SEQ ID NO: 102)
KU	ttctgtcgtacttatagatcgctacgttattcaa tttgaaaatctga (SEQ ID NO: 145)	gtcctgggagtgcgga (SEQ ID NO: 103)
KU	ttctgtcgtacttatagatcgctacgttattcaa tttgaaaatctgag (SEQ ID NO: 146)	tcctgggagtgcgga (SEQ ID NO: 104)
KU	ttctgtcgtacttatagatcgctacgttattcaa tttgaaaatctgagt (SEQ ID NO: 147)	cctgggagtgcgga (SEQ ID NO: 105)
KU	ttctgtcgtacttatagatcgctacgttattcaa tttgaaaatctgagtc (SEQ ID NO: 148)	ctgggagtgcgga (SEQ ID NO: 106)
Sm7	aat	tttgga
Sm7	aatt	tttgga
Sm7	aattt	ttgga
Sm7	aatttt	tgga
Sm7	aattttt	gga
COM	ctga	atgcctgcgagcatc (SEQ ID NO: 149)
COM	ctgaa	tgccctgcgagcatc (SEQ ID NO: 150)
COM	ctgaat	gcctgcgagcatc (SEQ ID NO: 151)
COM	ctgaatg	cctgcgagcatc (SEQ ID NO: 152)
COM	ctgaatgc	ctgcgagcatc (SEQ ID NO: 153)

COM	ctgaatgcc	tgcgagcatc (SEQ ID NO: 154)
COM	ctgaatgcct (SEQ ID NO: 155)	gcgagcatc
COM	ctgaatgcctg (SEQ ID NO: 156)	cgagcatc
COM	ctgaatgcctgc (SEQ ID NO: 157)	gagcatc
COM	ctgaatgcctgcg (SEQ ID NO: 158)	agcatc
COM	ctgaatgcctgcga (SEQ ID NO: 159)	gcatc
COM	ctgaatgcctgcgag (SEQ ID NO: 160)	catc
COM	ctgaatgcctgcgagc (SEQ ID NO: 161)	atc
PP7	ataag	gagtttatatggaaaccctta (SEQ ID NO: 162)
PP7	ataagg	agtttatatggaaaccctta (SEQ ID NO: 163)
PP7	ataagga	gttttatatggaaaccctta (SEQ ID NO: 164)
PP7	ataaggag	tttatatggaaaccctta (SEQ ID NO: 165)
PP7	ataaggagt	tttatatggaaaccctta (SEQ ID NO: 166)
PP7	Ataaggagtt (SEQ ID NO: 174)	tatatggaaaccctta (SEQ ID NO: 167)
PP7	Ataaggagttt (SEQ ID NO: 175)	atatggaaaccctta (SEQ ID NO: 168)
PP7	Ataaggagtta (SEQ ID NO: 176)	tatggaaaccctta (SEQ ID NO: 169)
PP7	Ataaggagttat (SEQ ID NO: 177)	atggaaaccctta (SEQ ID NO: 170)
PP7	Ataaggagttata (SEQ ID NO: 178)	tggaaaccctta (SEQ ID NO: 171)
PP7	ataaggagtttatat (SEQ ID NO: 179)	ggaaaccctta (SEQ ID NO: 172)
PP7	ataaggagtttatatg (SEQ ID NO: 180)	gaaaccctta (SEQ ID NO: 173)
PP7	ataaggagtttatatgg (SEQ ID NO: 181)	aaaccctta
PP7	Ataaggagtttatatgga (SEQ ID NO: 182)	aaccctta
PP7	Ataaggagtttatatggaa (SEQ ID NO: 183)	accctta
PP7	Ataaggagtttatatggaaa (SEQ ID NO: 184)	ccctta
PP7	Ataaggagtttatatggaaac (SEQ ID NO: 185)	cctta

The first part of the recruiting RNA motif and the second part of the recruiting RNA motif form a functional recruiting RNA motif after circularization.

The term “recruiting RNA motif” is defined above. The term “functional recruiting RNA motif”, as used herein, refers to a recruiting RNA motif that is capable of (specifically) binding an RNA binding domain. The term “RNA binding domain” is also defined above. The advantage of this circularization approach is that the recruiting RNA motif only becomes functional after circularization, as it is completed through the circularization. Thus, only circular RNA is packaged into the virus like particle (VLP). This increases the quality of the circular RNA delivery approach, as described herein.

The term “vector”, as used herein, refers to a structure to transfer genetic material into cells. The term “vector”, as used herein, encompasses viral and plasmid vectors. Plasmids with specially-constructed features are commonly used in laboratory for cloning purposes. These plasmids are generally non-conjugative but may have many more features, notably a multiple cloning site where multiple restriction enzyme cleavage sites allow for the insertion of a transgene insert. Plasmids may be used specifically as transcription vectors and such plasmids may lack crucial sequences for protein expression. Plasmids used for protein expression, called expression vectors, include elements for translation of proteins, such as a ribosome binding site, start and stop codons. Viral vectors are generally genetically engineered viruses carrying modified viral DNA or RNA that has been rendered non-infectious, but still contain viral promoters and also the transgene, thus, allowing for translation of the transgene through a viral promoter. However, because viral vectors frequently are lacking infectious sequences, they require helper viruses or packaging lines for large-scale transfection. A baculovirus may be used as viral vector.

Residues in two or more polypeptides or polynucleotides are said to “correspond” to each other if the residues occupy an analogous position in the polypeptide or polynucleotide structures. It is well known in the art that analogous positions in two or more polypeptides or polynucleotides can be determined by aligning the polypeptide or polynucleotide sequences based on amino acid sequence or nucleotide sequence similarities. Such alignment tools are well known to the person skilled in the art and can be, for example, obtained on the World Wide Web, e.g., ClustalW (www.ebi.ac.uk/clustalw) or Align (<http://www.ebi.ac.uk/emboss/align/index.html>) using standard settings, preferably for Align EMBOSS: needle, Matrix: Blosum62, Gap Open 10.0, Gap Extend 0.5.

The term “naturally occurring”, as used herein, as applied to an object refers to the fact that an object can be found in nature. For example, a polypeptide or polynucleotide sequence

that is present in an organism (including viruses) that can be isolated from a source in nature and which has not been intentionally modified by man in the laboratory is naturally occurring.

The term “artificial”, as used herein, as applied to an object refers to the fact that an object cannot be found in nature. For example, a polypeptide or polynucleotide sequence that is present in an organism (including viruses) that can be isolated from a source in nature and which has been intentionally modified by man in the laboratory is artificial. A polypeptide or polynucleotide sequence generated by man in the laboratory, e.g. chemically synthesized, is also artificial.

The term “treatment”, in particular “therapeutic treatment”, as used herein, refers to any therapy which improves the health status and/or prolongs (increases) the lifespan of a subject suffering from a disease. Said therapy may eliminate the disease in a subject, arrest or slow the development of the disease in a subject, inhibit the development of the disease in a subject, decrease the severity of symptoms in a subject suffering the disease, and/or decrease the recurrence in a subject who currently has or who previously has had a disease.

Preferably, the treatment is selected from the group consisting of gene therapy, cell therapy, cancer therapy, and the treatment of a disease such as an infectious disease, e.g. caused by bacteria or viruses such as SARS-CoV-2.

The present invention relates to the use of a virus like particle (VLP), as described herein, as medicament. The term “medicament”, as used herein, refers to a substance used in therapy, i.e. in treating, ameliorating or preventing a disease or disorder. In particular, the present invention relates to a virus like particle (VLP), as described herein, for use in therapy. Alternatively, the present invention relates a virus like particle (VLP), as described herein, for use in vaccination. In this respect, it should be noted that the active component is usually not the VLP itself, but the RNA element of interest being part of the circular RNA molecule comprised therein.

The term “vaccine”, as used herein, refers to an agent that can be used to elicit protective immunity in a recipient, e.g. human or animal recipient. To be effective, a vaccine can elicit immunity in a portion of the immunized population, as some individuals may fail to mount a robust or protective immune response or, in some cases, any immune response. This inability may stem from the genetic background of the recipient or because of an immunodeficiency condition (either acquired or congenital) or immunosuppression (e.g., due to treatment with chemotherapy or use of immunosuppressive drugs). Vaccine efficacy can be established in animal models.

The term “vaccination”, as used herein, means that a recipient, e.g. human or animal recipient, is challenged with a vaccine to induce a specific immunity. In the context of the present invention, the recipient can be challenged with a virus like particle (VLP) which further comprises an RNA element of interest. In one embodiment, the RNA element of interest is a sequence against which a protective immunity is elicited.

As used herein, the expressions “is for administration” and “is to be administered” have the same meaning as “is prepared to be administered”. In other words, the statement that an active compound “is for administration” has to be understood in that said active compound has been formulated and made up into doses so that said active compound is in a state capable of exerting its therapeutic activity. In the context of the present invention, the virus like particle (VLP), as described herein, can be prepared for administration.

The terms “therapeutically effective amount” or “therapeutic amount” are intended to mean that amount of a drug or pharmaceutical agent that will elicit the biological or medical response of a tissue, a system, animal or human that is being sought by a researcher, veterinarian, medical doctor or other clinician. The dosage regimen utilizing the virus like particle (VLP), as described herein, can be selected by the skilled practitioner in accordance with a variety of factors including type, species, age, weight, body mass index, sex and medical condition of the subject; the severity of the condition to be treated; the potency of the compound chosen to be administered; the route of administration; the purpose of the administration; and the renal and hepatic function of the subject. In the context of the present invention, the virus like particle (VLP), as described herein, can be administered or prepared to be administered in a therapeutically effective/therapeutic amount.

The composition, in particular the pharmaceutical composition, comprising the virus like particle (VLP), as described herein, may comprise one or more excipient(s), diluent(s), and/or carrier(s), all of which are preferably pharmaceutically acceptable. The term “pharmaceutically acceptable”, as used herein, means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia, European Pharmacopeia (Ph. Eur.) or other generally recognized pharmacopeia for use in animals, and more particularly in humans.

The term “excipient”, as used herein, is intended to indicate all substances in a pharmaceutical composition which are not active ingredients such as binders, lubricants, thickeners, surface active agents, preservatives, emulsifiers, buffers, flavoring agents, or colorants.

The term “diluent”, as used herein, relates to a diluting and/or thinning agent. Moreover, the term “diluent” includes a solution, suspension (e.g. liquid or solid suspension) and/or media.

The term “carrier”, as used herein, relates to one or more compatible solid or liquid fillers, which are suitable for an administration, e.g. to a human. The term “carrier” relates to a natural or synthetic organic or inorganic component which is combined with an active component in order to facilitate the application of the active component, or the permeation of the active component to the intended site of action. Preferably, carrier components are sterile liquids such as water or oils, including those which are derived from mineral oil, animals, or plants, such as peanut oil, soy bean oil, sesame oil, sunflower oil, etc. Salt solutions and aqueous dextrose and glycerin solutions may also be used as aqueous carrier compounds. A carrier compound that improves or facilitates permeation of the skin in topical applications is dimethyl sulfoxide (DMSO). Another preferred carrier consists of layered double hydroxide (LDH) nanoparticles. For example, such an LDH nanoparticles can be of the form $[Mg_3Al(OH)_8](CH_3CHOHCOO)$, that can be obtained, for example, by a reaction of $Mg(lactate)_2 \cdot 3H_2O$ and $Al(lactate)_3$ in NaOH-controlled pH at 45-65 °C and protected from CO_2 and carbonic acids. A circularized RNA may intercalate into LDH nanoparticles and can be administered as LDH nanoparticle or be co-administered with VLPs.

Pharmaceutically acceptable carriers or diluents for therapeutic use are well known in the pharmaceutical art, and are described, for example, in Remington's Pharmaceutical Sciences, Mack Publishing Co. (A. R. Gennaro edit. 1985). Examples of suitable carriers include, for example, magnesium carbonate, magnesium stearate, talc, lactose, pectin, dextrin, starch, gelatin, tragacanth, methylcellulose, sodium carboxymethylcellulose, a low melting wax, cocoa butter, and the like. Examples of suitable diluents include ethanol, glycerol, and water.

Pharmaceutical carriers, diluents, and/or excipients can be selected with regard to the intended route of administration and standard pharmaceutical practice. The pharmaceutical compositions of the present invention may comprise as, or in addition to, the carrier(s), excipient(s) or diluent(s) any suitable binder(s), lubricant(s), suspending agent(s), coating agent(s), and/or solubilising agent(s). Examples of suitable binders include starch, gelatin, natural sugars such as glucose, lactose, sucrose, trehalose, corn sweeteners, natural and synthetic gums, such as acacia, tragacanth or sodium alginate, carboxymethyl cellulose, and polyethylene glycol. Examples of suitable lubricants include sodium oleate, sodium stearate, magnesium stearate, sodium benzoate, sodium acetate, sodium chloride, and the like. Preservatives, stabilizers, dyes, and even flavoring agents may be provided in the

pharmaceutical composition. Examples of preservatives include sodium benzoate, sorbic acid, and esters of p-hydroxybenzoic acid. Antioxidants and suspending agents may be also used.

The term “subject”, as used herein, refers to any individual which may receive the virus like particle (VLP), as described herein. The term “subject”, as used herein, refers to any individual that/who may benefit from the treatment with the virus like particle (VLP), as described herein.

The subject may be a vertebrate, e.g. a human being, dog, cat, sheep, goat, cow, horse, camel or pig. It is particularly preferred that the “subject” is a human being.

The terms “subject”, “individual”, or “patient” are used interchangeably herein.

Embodiments of the invention

The present invention will now be further described. In the following passages, different aspects of the invention are defined in more detail. Each aspect so defined may be combined with any other aspect or aspects unless clearly indicated to the contrary. In particular, any feature indicated as being preferred or advantageous may be combined with any other feature or features indicated as being preferred or advantageous, unless clearly indicated to the contrary.

The present inventors surprisingly found that circular RNAs (circRNAs) can be packaged into virus like particles (VLPs). By incorporating recruiting RNA motifs into the molecule to be packaged, circRNAs such as coding or non-coding circRNAs can be selectively packaged into VLPs. The recruiting RNA motifs only become functional through circularization, whereby the molecule to be packaged is packaged exclusively in its circularized state. Surprisingly, these RNAs can also be packaged in the envelopes of DNA viruses. These VLPs are capable for *in vivo* or *in vitro* delivery of (therapeutic) circRNA molecules.

Thus, in a first aspect, the present invention relates a (linear) polynucleotide (capable of circularization) comprising in the following order from 5' to 3':

a first backsplicing intron sequence,

a first part of a recruiting RNA motif,

a nucleotide sequence of interest,

a second part of a recruiting RNA motif, and

a second backsplicing intron sequence,

wherein the first part of the recruiting RNA motif and the second part of the recruiting RNA motif form a functional recruiting RNA motif after circularization.

The (linear) polynucleotide (capable of circularization) can be a DNA or an RNA (or mixed DNA and RNA) polynucleotide. Preferably, the (linear) polynucleotide is a DNA polynucleotide.

The (linear) polynucleotide (capable of circularization) can be a DNA or an RNA (or mixed DNA and RNA) polynucleotide that is capable of forming a circular structure in which the 5' and 3' termini are covalently linked with each other.

Alternatively, the (linear) polynucleotide (capable of circularization) can be a DNA or an RNA (or mixed DNA and RNA) polynucleotide that is capable of forming a circular structure in which the 5' and 3' termini associate with high stability in a non-covalent fashion.

Preferably, the (linear) polynucleotide (capable of circularization) is a DNA or an RNA (or mixed DNA and RNA) polynucleotide that is capable of forming a circular structure in which the 5' and 3' termini are covalently linked with each other.

Particularly, a circular polynucleotide occurs from the linear polynucleotide, specifically from an exon of the linear polynucleotide, by backsplicing when the 5' backsplicing intron sequence is joined to the upstream 3' backsplicing intron sequence.

The 5' and 3' termini, in particular the 5' and 3' termini of one of its exons, can be covalently linked with each other by joining the first backsplicing intron sequence to the second backsplicing intron sequence. Thousands of circRNAs have been shown to be expressed in eukaryotic cells. Public circRNA datasets and corresponding backsplicing intron sequences can be explored can be explored at circBase (<http://www.circbase.org/>), for example.

The backsplicing intron sequences are preferably introns containing a splice sites along with short inverted repeats, such as Alu elements, to allow protein-assisted circularization of exons. More preferably, the backsplicing intron sequence are from the ZKSCAN1 genes, e.g. human ZKSCAN1 genes. The ZKSCAN1 genes belong to a zinc finger family gene. Even more preferably, the first backsplicing intron sequence has a nucleotide sequence according to SEQ ID NO: 34, or is a nucleotide sequence having at least 80%, preferably at least 85%, more preferably at least 90%, and most preferably at least 95% or 99%, i.e. 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99%, sequence identity thereto and/or the second backsplicing intron sequence has a nucleotide sequence according to SEQ ID NO: 35, or is a nucleotide sequence having at least 80%, preferably at least 85%, more preferably at least 90%, and most preferably at least 95% or 99%, i.e. 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99%, sequence identity thereto.

Alternatively, the autocatalytic backsplicing intron sequences are preferably derived from group I or group II introns. More preferably, the autocatalytic backsplicing intron

sequences are derived from group I intron T4 from bacteriophage T4 td gene. The intron has been split and rearranged so that after transcription, a single internal exon is rendered circular by the group I splicing reaction. Even more preferably, the first autocatalytic backsplicing intron sequence has a nucleotide sequence according to SEQ ID NO: 43, or is a nucleotide sequence having at least 80%, preferably at least 85%, more preferably at least 90%, and most preferably at least 95% or 99%, i.e. 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99%, sequence identity thereto and/or the second backsplicing intron sequence has a nucleotide sequence according to SEQ ID NO: 44, or is a nucleotide sequence having at least 80%, preferably at least 85%, more preferably at least 90%, and most preferably at least 95% or 99%, i.e. 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99%, sequence identity thereto.

The nucleotide sequence of interest may be a DNA or an RNA sequence. The DNA sequence can encode an RNA element or a protein. The RNA element or protein encoded by the DNA sequence has a specific function and is, therefore, of interest. In case the nucleotide sequence of interest is an RNA sequence, the RNA sequence itself can exert a specific function as RNA element or can encode a protein. The function of the RNA element is, for example, the inhibition of the expression of genes, e.g. by application of antisense RNA. Alternatively, therapeutic active RNA may be applied to induce gene expression, e.g. to elicit an immune response. In one prominent example, the expression of the spike protein of SARS-CoV-2 is induced to elicit an immune response against the SARS-CoV-2 virus.

Preferably, the RNA element of interest is selected from the group consisting of coding or non-coding RNA. More preferably, the RNA element of interest is selected from the group consisting of a shRNA, miRNA, protein coding RNA, pre-miRNA, piRNA, RNA complementary to other non-coding RNA, and a ribozyme. Thus, the RNA element of interest may encode a product of interest. A product of interest may be a polypeptide or protein of interest. The RNA element of interest may also be a non-coding RNA, e.g. a miRNA. Non-coding RNAs are frequently used to silence a corresponding target gene.

In the (linear) polynucleotide (capable of circularization), the recruiting RNA motif is splitted into two parts, a first part of the recruiting RNA motif and a second part of the recruiting motif, in a way that the parts no longer have a significant recruiting function on their own. The first part of the recruiting RNA motif and the second part of the recruiting RNA motif form a functional recruiting RNA motif after circularization. Only a functional recruiting RNA motif is capable of (specifically) binding an RNA binding domain connected to/fused to/associated with the capsid protein of a virus or a fragment or a derivative thereof having capsid protein

function, preferably via a linker, and can, thus, be encapsidated/encapsulated within a virus like particle (VLP) as part of a circular RNA (circRNA) molecule, as described herein.

In one embodiment, the polynucleotide comprises between the first part of a recruiting RNA motif and the nucleotide sequence of interest an internal ribosomal entry site (IRES) or a N6-methyladenosine (m6a) for translation initiation. This is especially required in cases where the nucleotide sequence of interest encodes for a protein.

In one (further or additional) embodiment, the polynucleotide comprises at the 5' end a promoter and/or at the 3' end a poly A tail. This is especially required in cases where the nucleotide sequence of interest encodes for a protein.

In one preferred embodiment, the circularization of the (linear) polynucleotide, in particular the circularization of one of its exons of the (linear) polynucleotide, to a circular polynucleotide is achievable via protein-assisted backsplicing or autocatalytic backsplicing (see definition above).

In one another preferred embodiment, the polynucleotide does not contain an in frame stop codon to enable rolling circle translation after circularization. More than one protein can be translated by rolling circle translation if protease target sites, ribosomal shift or ribosomal skipping sequences are incorporated into the open reading frame. An example for ribosomal skipping sequence is the F2A (or 2A-like) sequence of the foot-and-mouth disease virus (FMDV). An example for ribosomal shift sequence is the leaky tandem termination/start codon with the sequence UGAUGA.

In one more preferred embodiment, the recruiting RNA motif is selected from the group consisting of a MS2 phage operator stem-loop, a telomerase Ku binding motif, a telomerase Sm7 binding motif, a SfMu phage COM stem-loop, and a PP7 phage operator stem-loop. For example, the recruiting motif can be splitted as follows.

	second part of the recruiting motif	first part of the recruiting RNA motif
MS2	gcgca	catgaggatcacccatgtgc (SEQ ID NO: 45)
MS2	gcgcac	atgaggatcacccatgtgc (SEQ ID NO: 46)
MS2	gcgcaca	tgaggatcacccatgtgc (SEQ ID NO: 47)
MS2	gcgcacat	taggatcacccatgtgc (SEQ ID NO: 48)
MS2	gcgcacatg	aggatcacccatgtgc (SEQ ID NO: 49)
MS2	gcgcacatga (SEQ ID NO: 56)	ggatcacccatgtgc (SEQ ID NO: 50)
MS2	gcgcacatgag (SEQ ID NO: 57)	gatcacccatgtgc (SEQ ID NO: 51)
MS2	gcgcacatgagg (SEQ ID NO: 58)	atcacccatgtgc (SEQ ID NO: 52)

MS2	gcgcacatgagga (SEQ ID NO: 59)	tcacccatgtgc (SEQ ID NO: 53)
MS2	gcgcacatgaggat (SEQ ID NO: 60)	cacccatgtgc (SEQ ID NO: 54)
MS2	gcgcacatgaggatc (SEQ ID NO: 61)	acccatgtgc (SEQ ID NO: 55)
MS2	gcgcacatgaggatca (SEQ ID NO: 62)	cccatgtgc
MS2	gcgcacatgaggatcac (SEQ ID NO: 63)	ccatgtgc
MS2	gcgcacatgaggatcacc (SEQ ID NO: 64)	catgtgc
KU	ttctgtcgtac (SEQ ID NO: 107)	ttatagatcgctacgttatttcaattttgaaaatctgagtcctggga gtgcgga (SEQ ID NO: 65)
KU	ttctgtcgtact (SEQ ID NO: 108)	tatagatcgctacgttatttcaattttgaaaatctgagtcctggga gtgcgga (SEQ ID NO: 66)
KU	ttctgtcgtactt (SEQ ID NO: 109)	atagatcgctacgttatttcaattttgaaaatctgagtcctgggag tgcgga (SEQ ID NO: 67)
KU	ttctgtcgtactta (SEQ ID NO: 110)	tagatcgctacgttatttcaattttgaaaatctgagtcctgggagt gcgga (SEQ ID NO: 68)
KU	ttctgtcgtacttat (SEQ ID NO: 111)	agatcgctacgttatttcaattttgaaaatctgagtcctgggagt cgga (SEQ ID NO: 69)
KU	ttctgtcgtacttata (SEQ ID NO: 112)	gatcgctacgttatttcaattttgaaaatctgagtcctgggagtgc gga (SEQ ID NO: 70)
KU	ttctgtcgtacttatag (SEQ ID NO: 113)	atcgctacgttatttcaattttgaaaatctgagtcctgggagtgc ga (SEQ ID NO: 71)
KU	ttctgtcgtacttataga (SEQ ID NO: 114)	tcgctacgttatttcaattttgaaaatctgagtcctgggagtgcg ga (SEQ ID NO: 72)
KU	ttctgtcgtacttatagat (SEQ ID NO: 115)	cgctacgttatttcaattttgaaaatctgagtcctgggagtgcg ga (SEQ ID NO: 73)
KU	ttctgtcgtacttatagatc (SEQ ID NO: 116)	gctacgttatttcaattttgaaaatctgagtcctgggagtgcgga (SEQ ID NO: 74)
KU	ttctgtcgtacttatagatcg (SEQ ID NO: 117)	ctacgttatttcaattttgaaaatctgagtcctgggagtgcgga (SEQ ID NO: 75)
KU	ttctgtcgtacttatagatcgc (SEQ ID NO: 118)	tacgttatttcaattttgaaaatctgagtcctgggagtgcgga (SEQ ID NO: 76)

KU	ttcttgctgacttatagatcgct (SEQ ID NO: 119)	acgttattcaattttgaaaatctgagtcctgggagtgcgga (SEQ ID NO: 77)
KU	ttcttgctgacttatagatcgcta (SEQ ID NO: 120)	cggttattcaattttgaaaatctgagtcctgggagtgcgga (SEQ ID NO: 78)
KU	ttcttgctgacttatagatcgctac (SEQ ID NO: 121)	gttatttcaattttgaaaatctgagtcctgggagtgcgga (SEQ ID NO: 79)
KU	ttcttgctgacttatagatcgctacg (SEQ ID NO: 122)	ttatttcaattttgaaaatctgagtcctgggagtgcgga (SEQ ID NO: 80)
KU	ttcttgctgacttatagatcgctacgt (SEQ ID NO: 123)	tatttcaattttgaaaatctgagtcctgggagtgcgga (SEQ ID NO: 81)
KU	ttcttgctgacttatagatcgctacgtt (SEQ ID NO: 124)	atttcaattttgaaaatctgagtcctgggagtgcgga (SEQ ID NO: 82)
KU	ttcttgctgacttatagatcgctacgtta (SEQ ID NO: 125)	tttcaattttgaaaatctgagtcctgggagtgcgga (SEQ ID NO: 83)
KU	ttcttgctgacttatagatcgctacgttat (SEQ ID NO: 126)	ttcaattttgaaaatctgagtcctgggagtgcgga (SEQ ID NO: 84)
KU	ttcttgctgacttatagatcgctacgttatt (SEQ ID NO: 127)	tcaattttgaaaatctgagtcctgggagtgcgga (SEQ ID NO: 85)
KU	ttcttgctgacttatagatcgctacgttattt (SEQ ID NO: 128)	caattttgaaaatctgagtcctgggagtgcgga (SEQ ID NO: 86)
KU	ttcttgctgacttatagatcgctacgttatttc (SEQ ID NO: 129)	aattttgaaaatctgagtcctgggagtgcgga (SEQ ID NO: 87)
KU	ttcttgctgacttatagatcgctacgttatttca (SEQ ID NO: 130)	attttgaaaatctgagtcctgggagtgcgga (SEQ ID NO: 88)
KU	ttcttgctgacttatagatcgctacgttatttcaa (SEQ ID NO: 131)	ttttgaaaatctgagtcctgggagtgcgga (SEQ ID NO: 89)
KU	ttcttgctgacttatagatcgctacgttatttcaat (SEQ ID NO: 132)	tttgaaaatctgagtcctgggagtgcgga (SEQ ID NO: 90)
KU	ttcttgctgacttatagatcgctacgttatttcaatt (SEQ ID NO: 133)	ttgaaaatctgagtcctgggagtgcgga (SEQ ID NO: 91)
KU	ttcttgctgacttatagatcgctacgttatttcaattt (SEQ ID NO: 134)	tgaaaatctgagtcctgggagtgcgga (SEQ ID NO: 92)
KU	ttcttgctgacttatagatcgctacgttatttcaatttt (SEQ ID NO: 135)	gaaaatctgagtcctgggagtgcgga (SEQ ID NO: 93)

KU	ttcttgctgacttatagatcgctacgttattcaa ttttg (SEQ ID NO: 136)	aaaatctgagtcctgggagtgcgga (SEQ ID NO: 94)
KU	ttcttgctgacttatagatcgctacgttattcaa ttttga (SEQ ID NO: 137)	aaatctgagtcctgggagtgcgga (SEQ ID NO: 95)
KU	ttcttgctgacttatagatcgctacgttattcaa ttttgaa (SEQ ID NO: 138)	aatctgagtcctgggagtgcgga (SEQ ID NO: 96)
KU	ttcttgctgacttatagatcgctacgttattcaa ttttgaaa (SEQ ID NO: 139)	atctgagtcctgggagtgcgga (SEQ ID NO: 97)
KU	ttcttgctgacttatagatcgctacgttattcaa ttttgaaaa (SEQ ID NO: 140)	tctgagtcctgggagtgcgga (SEQ ID NO: 98)
KU	ttcttgctgacttatagatcgctacgttattcaa ttttgaaaat (SEQ ID NO: 141)	ctgagtcctgggagtgcgga (SEQ ID NO: 99)
KU	ttcttgctgacttatagatcgctacgttattcaa ttttgaaaatc (SEQ ID NO: 142)	tgagtcctgggagtgcgga (SEQ ID NO: 100)
KU	ttcttgctgacttatagatcgctacgttattcaa ttttgaaaatct (SEQ ID NO: 143)	gagtcctgggagtgcgga (SEQ ID NO: 101)
KU	ttcttgctgacttatagatcgctacgttattcaa ttttgaaaatctg (SEQ ID NO: 144)	agtcctgggagtgcgga (SEQ ID NO: 102)
KU	ttcttgctgacttatagatcgctacgttattcaa ttttgaaaatctga (SEQ ID NO: 145)	gtcctgggagtgcgga (SEQ ID NO: 103)
KU	ttcttgctgacttatagatcgctacgttattcaa ttttgaaaatctgag (SEQ ID NO: 146)	tcctgggagtgcgga (SEQ ID NO: 104)
KU	ttcttgctgacttatagatcgctacgttattcaa ttttgaaaatctgagt (SEQ ID NO: 147)	cctgggagtgcgga (SEQ ID NO: 105)
KU	ttcttgctgacttatagatcgctacgttattcaa ttttgaaaatctgagtc (SEQ ID NO: 148)	ctgggagtgcgga (SEQ ID NO: 106)
Sm7	aat	ttttgga
Sm7	aatt	tttgga
Sm7	aattt	ttgga
Sm7	aatttt	tgga
Sm7	aattttt	gga
COM	ctga	atgcctgcgagcatc (SEQ ID NO: 149)
COM	ctgaa	tgccctgcgagcatc (SEQ ID NO: 150)

COM	ctgaat	gcctgcgagcatc (SEQ ID NO: 151)
COM	ctgaatg	cctgcgagcatc (SEQ ID NO: 152)
COM	ctgaatgc	ctgcgagcatc (SEQ ID NO: 153)
COM	ctgaatgcc	tgcgagcatc (SEQ ID NO: 154)
COM	ctgaatgcct (SEQ ID NO: 155)	gcgagcatc
COM	ctgaatgcctg (SEQ ID NO: 156)	cgagcatc
COM	ctgaatgcctgc (SEQ ID NO: 157)	gagcatc
COM	ctgaatgcctgcg (SEQ ID NO: 158)	agcatc
COM	ctgaatgcctgcga (SEQ ID NO: 159)	gcatc
COM	ctgaatgcctgcgag (SEQ ID NO: 160)	catc
COM	ctgaatgcctgcgagc (SEQ ID NO: 161)	atc
PP7	ataag	gagtttatatggaaaccctta (SEQ ID NO: 162)
PP7	ataagg	agtttatatggaaaccctta (SEQ ID NO: 163)
PP7	ataagga	gtttatatggaaaccctta (SEQ ID NO: 164)
PP7	ataaggag	tttatatggaaaccctta (SEQ ID NO: 165)
PP7	ataaggagt	tttatatggaaaccctta (SEQ ID NO: 166)
PP7	Ataaggagtt (SEQ ID NO: 174)	tatatggaaaccctta (SEQ ID NO: 167)
PP7	Ataaggagttt (SEQ ID NO: 175)	atatggaaaccctta (SEQ ID NO: 168)
PP7	Ataaggagttta (SEQ ID NO: 176)	tatggaaaccctta (SEQ ID NO: 169)
PP7	Ataaggagtttat (SEQ ID NO: 177)	atggaaaccctta (SEQ ID NO: 170)
PP7	Ataaggagtttata (SEQ ID NO: 178)	tggaaccctta (SEQ ID NO: 171)
PP7	ataaggagtttatat (SEQ ID NO: 179)	ggaaaccctta (SEQ ID NO: 172)
PP7	ataaggagtttatatg (SEQ ID NO: 180)	gaaaccctta (SEQ ID NO: 173)
PP7	ataaggagtttatatgg (SEQ ID NO: 181)	aaaccctta
PP7	Ataaggagtttatatgga (SEQ ID NO: 182)	aaccctta
PP7	Ataaggagtttatatggaa (SEQ ID NO: 183)	accctta

PP7	Ataaggagtttatatggaaa (SEQ ID NO: 184)	ccctta
PP7	Ataaggagtttatatggaaac (SEQ ID NO: 185)	ccctta

The (linear) polynucleotide (capable of circularization) has preferably a length of between 100 and 50.000 nucleotides, has more preferably a length of between 100 and 5.000 nucleotides, and has even more preferably a length of between 100 and 2.000 nucleotides.

In a second aspect, the present invention relates to a vector comprising the polynucleotide according to the first aspect.

In a third aspect, the present invention relates to an artificial circular RNA molecule comprising an RNA element of interest and a recruiting RNA motif.

The artificial circular RNA molecule comprising an RNA element of interest and a recruiting RNA motif may be produced from the (linear) polynucleotide (which is capable of circularization) according to the first aspect.

The artificial circular RNA molecule can be a type of single-stranded RNA which, unlike linear RNA, forms a covalently closed continuous loop. Alternatively, the artificial circular RNA molecule can be a type of single-stranded RNA which, unlike linear RNA, forms a non-covalently closed continuous loop (see first aspect).

The RNA element of interest has a specific function or is of a specific purpose, and is, therefore, of interest. The function of the RNA element of interest is, for example, the inhibition of the expression of genes, e.g. by application of antisense RNA. Alternatively, therapeutic active RNA may be applied to induce gene expression, e.g. to elicit an immune response.

The RNA element of interest may be a coding or a non-coding RNA element.

Preferably, the RNA element of interest is selected from the group consisting of a shRNA, miRNA, protein coding RNA, pre-miRNA, piRNA, RNA complementary to other non-coding RNA, and a ribozyme.

The recruiting RNA motif is, together with the RNA element of interest, part of the artificial circular RNA molecule. Specifically, the recruiting RNA motif comprised in the artificial circular RNA molecule is capable of (specifically) binding to an RNA binding domain connected to/fused to/associated with a capsid protein of a virus or a fragment or a derivative thereof having capsid protein function, preferably via a linker. In this way, the artificial circular RNA molecule can be encapsidated/encapsulated within the capsid protein structure of a virus like particle.

Preferably, the recruiting RNA motif is selected from the group consisting of a MS2 phage operator stem-loop, a telomerase Ku binding motif, a telomerase Sm7 binding motif, a SfMu phage COM stem-loop, and a PP7 phage operator stem-loop.

More preferably, the recruiting RNA motif has a nucleotide sequence selected from the group consisting of SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15 (or is a nucleotide sequence transcribed thereof), and a nucleotide sequence having at least 80%, preferably at least 85%, more preferably at least 90%, and most preferably at least 95% or 99%, i.e. 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99%, sequence identity thereto.

A recruiting RNA motif having at least 80%, preferably at least 85%, more preferably at least 90%, and most preferably at least 95% or 99%, i.e. 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99%, sequence identity to the nucleotide sequence selected from the group consisting of SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15 (or a nucleotide sequence transcribed thereof) can be designated as a recruiting RNA motif variant. Such a recruiting RNA motif variant is still able to (specifically) bind to the RNA binding domain as described above. The experimental section provides, for example, sufficient information in this respect.

Specifically, the recruiting RNA motif selected from the group consisting of a MS2 phage operator stem-loop, a telomerase Ku binding motif, a telomerase Sm7 binding motif, a SfMu phage COM stem-loop, and a PP7 phage operator stem-loop is together with the RNA element of interest selected from the group consisting of a shRNA, miRNA, protein coding RNA, pre-miRNA, piRNA, RNA complementary to other non-coding RNA, and a ribozyme part of/comprised in the artificial circular RNA molecule.

As mentioned above, the recruiting RNA motif comprised in the artificial circular RNA molecule is capable of (specifically) binding to an RNA binding domain connected to/fused to/associated with a capsid protein of a virus or a fragment or a derivative thereof having capsid protein function, preferably via a linker.

Preferably, the at least one RNA binding domain is selected from the group consisting of a MS2 coat protein or an RNA-binding section thereof, a Ku protein or an RNA-binding section thereof, a Sm7 protein or an RNA-binding section thereof, a SfMu phage COM RNA binding protein or an RNA-binding section thereof, and a PP7 coat protein or an RNA-binding section thereof.

More preferably, the at least one RNA binding domain has an amino acid sequence selected from the group consisting of SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 12,

SEQ ID NO: 14, SEQ ID NO: 16, and an amino acid sequence having at least 80%, preferably at least 85%, more preferably at least 90%, and most preferably at least 95% or 99%, i.e. 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99%, sequence identity thereto.

An RNA binding domain having at least 80%, preferably at least 85%, more preferably at least 90%, and most preferably at least 95% or 99%, i.e. 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99%, sequence identity to the amino acid sequence selected from the group consisting of SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16 can be designated as an RNA binding domain variant. Such an RNA binding domain variant is still able to (specifically) bind to the recruiting RNA motif as described above. The experimental section provides, for example, sufficient information in this respect.

In one preferred embodiment,

- (i) the at least one RNA binding domain (to which the recruiting RNA motif is capable of binding) is a MS2 coat protein or an RNA-binding section thereof and the recruiting RNA motif is a MS2 phage operator stem-loop,
- (ii) the at least one RNA binding domain (to which the recruiting RNA motif is capable of binding) is a Ku protein or an RNA-binding section thereof and the recruiting RNA motif is a telomerase Ku binding motif,
- (iii) the at least one RNA binding domain (to which the recruiting RNA motif is capable of binding) is a Sm7 protein or an RNA-binding section thereof and the recruiting RNA motif is a telomerase Sm7 binding motif,
- (iv) the at least one RNA binding domain (to which the recruiting RNA motif is capable of binding) is a SfMu phage COM RNA binding protein or an RNA-binding section thereof and the recruiting RNA motif is a SfMu phage COM stem-loop, and/or
- (v) the at least one RNA binding domain (to which the recruiting RNA motif is capable of binding) is a PP7 coat protein or an RNA-binding section thereof and the recruiting RNA motif is PP7 phage operator stem-loop.

In one more preferred embodiment,

the at least one RNA binding domain (to which recruiting RNA motif is capable of binding) has an amino acid sequence selected from the group consisting of SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, and an amino acid sequence having at least 80%, preferably at least 85%, more preferably at least 90%, and most

preferably at least 95% or 99%, i.e. 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99%, sequence identity thereto, and/or

the recruiting RNA motif has a nucleotide sequence selected from the group consisting of SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15 (or is a nucleotide sequence transcribed thereof), and a nucleotide sequence having at least 80%, preferably at least 85%, more preferably at least 90%, and most preferably at least 95% or 99%, i.e. 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99%, sequence identity thereto.

The artificial circular RNA molecule has preferably a length of between 50 and 50.000 nucleotides, has more preferably a length of between 50 and 5.000 nucleotides, and has even more preferably a length of between 50 and 2.000 nucleotides.

In a fourth aspect, the present invention relates to a complex comprising or to a complex of

- (i) a polypeptide comprising a capsid protein of a virus or a fragment or a derivative thereof having capsid protein function and at least one RNA binding domain (e.g. 1, 2, or 3 RNA binding domain(s)), and
- (ii) a (an artificial) circular RNA molecule comprising an RNA element of interest.

Specifically, the circular RNA molecule is an artificial circular RNA molecule, preferably the artificial circular RNA molecule according to the third aspect.

In one embodiment, the at least one RNA binding domain is connected to/fused to/associated with the capsid protein of a virus or a fragment or a derivative thereof having capsid protein function, preferably via a linker.

In one preferred embodiment, the at least one RNA binding domain is connected to/fused to/associated with the N-terminus and/or C-terminus of the capsid protein of a virus or a fragment or a derivative thereof having capsid protein function, preferably via a linker.

Any linker for coupling the capsid protein of a virus or the fragment or the derivative thereof having capsid protein function with the at least one RNA binding domain may be used. In an exemplarily embodiment, the linker has an amino acid sequence according to SEQ ID NO: 5.

Specifically, the polypeptide comprising a capsid protein of a virus or a fragment or a derivative thereof having capsid protein function and at least one RNA binding domain is present/produced as a fusion polypeptide. Especially, the polypeptide comprising a capsid protein of a virus or a fragment or a derivative thereof having capsid protein function and at least one RNA binding domain, wherein the capsid protein of a virus or a fragment or a derivative thereof having capsid protein function is connected to the at least one RNA binding domain via a linker is present/produced as a fusion polypeptide.

The at least one RNA binding domain may be a naturally occurring RNA binding domain or an artificial RNA binding domain.

Preferably, the at least one RNA binding domain is selected from the group consisting of a MS2 coat protein or an RNA-binding section thereof, a Ku protein or an RNA-binding section thereof, a Sm7 protein or an RNA-binding section thereof, a SfMu phage COM RNA binding protein or an RNA-binding section thereof, and a PP7 coat protein or an RNA-binding section thereof. Thus, it is preferred that the polypeptide comprises a capsid protein of a virus or a fragment or a derivative thereof having capsid protein function and at least one RNA binding domain, wherein the at least one RNA binding domain is selected from the group consisting of a MS2 coat protein or an RNA-binding section thereof, a Ku protein or an RNA-binding section thereof, a Sm7 protein or an RNA-binding section thereof, a SfMu phage COM RNA binding protein or an RNA-binding section thereof, and a PP7 coat protein or an RNA-binding section thereof. The RNA binding domain may be attached to the capsid protein of a virus or a fragment or a derivative thereof having capsid protein function via a linker or not.

As mentioned above, the (artificial) circular RNA molecule comprises an RNA element of interest. In one embodiment, the (artificial) circular RNA molecule comprising an RNA element of interest further comprises a recruiting RNA motif. The recruiting RNA motif comprised in the artificial circular RNA molecule (specifically) binds to the RNA binding domain connected to/fused to/associated with a capsid protein of a virus or a fragment or a derivative thereof having capsid protein function. In this way, the artificial circular RNA molecule forms a complex with the capsid protein structure.

The RNA element of interest has a specific function or is of a specific purpose, and is, therefore, of interest. The function of the RNA element of interest is, for example, the inhibition of the expression of genes, e.g. by application of antisense RNA. Alternatively, therapeutic active RNA may be applied to induce gene expression, e.g. to elicit an immune response.

The RNA element of interest may be a coding or a non-coding RNA element.

Preferably, the RNA element of interest is selected from the group consisting of a shRNA, miRNA, protein encoding RNA, pre-miRNA, piRNA, RNA complementary to other non-coding RNA, and a ribozyme.

Preferably, the recruiting RNA motif is selected from the group consisting of a MS2 phage operator stem-loop, a telomerase Ku binding motif, a telomerase Sm7 binding motif, a SfMu phage COM stem-loop, and a PP7 phage operator stem-loop.

More preferably, the RNA element of interest is selected from the group consisting of a shRNA, miRNA, protein encoding RNA, pre-miRNA, piRNA, RNA complementary to other non-

coding RNA, and a ribozyme and the recruiting RNA motif is selected from the group consisting of a MS2 phage operator stem-loop, a telomerase Ku binding motif, a telomerase Sm7 binding motif, a SfMu phage COM stem-loop, and a PP7 phage operator stem-loop.

In one preferred embodiment,

- (i) the at least one RNA binding domain comprised in the polypeptide is a MS2 coat protein or an RNA-binding section thereof and the recruiting RNA motif (to which the at least one RNA binding domain binds) is a MS2 phage operator stem-loop,
- (ii) the at least one RNA binding domain comprised in the polypeptide is a Ku protein or an RNA-binding section thereof and the recruiting RNA motif (to which the at least one RNA binding domain binds) is a telomerase Ku binding motif,
- (iii) the at least one RNA binding domain comprised in the polypeptide is a Sm7 protein or an RNA-binding section thereof and the recruiting RNA motif (to which the at least one RNA binding domain binds) is a telomerase Sm7 binding motif,
- (iv) the at least one RNA binding domain comprised in the polypeptide is a SfMu phage COM RNA binding protein or an RNA-binding section thereof and the recruiting RNA motif (to which the at least one RNA binding domain binds) is a SfMu phage COM stem-loop, and/or
- (v) the at least one RNA binding domain comprised in the polypeptide is a PP7 coat protein or an RNA-binding section thereof and the recruiting RNA motif (to which the at least one RNA binding domain binds) is PP7 phage operator stem-loop.

In one more preferred embodiment,

the at least one RNA binding domain has an amino acid sequence selected from the group consisting of SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, and an amino acid sequence having at least 80%, preferably at least 85%, more preferably at least 90%, and most preferably at least 95% or 99%, i.e. 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99%, sequence identity thereto, and/or the recruiting RNA motif has a nucleotide sequence selected from the group consisting of SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15 (or is a nucleotide sequence transcribed thereof), and a nucleotide sequence having at least 80%, preferably at least 85%, more preferably at least 90%, and most preferably at least 95% or 99%, i.e. 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99%, sequence identity thereto.

The virus may be a DNA or RNA virus.

In one further embodiment,

the DNA virus is selected from the group consisting of a papovavirus, a papilloma virus, a polyoma virus, an adenovirus, and a parvovirus virus, or
the RNA virus is a retrovirus, a norovirus, or an orthomyxovirus.

In one preferred embodiment,

the polyoma virus is a Simian Virus 40 (SV40), a BK virus (BKV), a JC virus (JCV), or a MPy virus (MPyV),

the parvovirus is an adeno-associated virus (AAV),

the retrovirus is a lentivirus,

the norovirus is a Norwalk virus, or

the orthomyxovirus is an influenza A or influenza B virus.

The capsid protein may be a capsid protein of an RNA or a DNA virus.

In one another embodiment,

the capsid protein of a DNA virus is selected from the group consisting of a papovavirus capsid protein, a papilloma virus capsid protein, a polyoma virus capsid protein, an adenovirus capsid protein, and a parvovirus capsid protein, or

the capsid protein of an RNA virus is a lentivirus capsid protein.

In one preferred embodiment,

- (i) the parvovirus capsid protein is an adeno-associated virus capsid protein, preferably an AAV9 capsid protein,
- (ii) the polyoma virus capsid protein is a SV40 capsid protein, a BKV capsid protein, a JCV capsid protein, or a MPyV capsid protein, or
- (iii) the lentivirus capsid protein is a nucleocapsid (NC) protein, a matrix protein (MA) or a capsid protein (CA).

In one even more preferred embodiment,

- (i) the AAV9 capsid protein is an AAV9 VP1 capsid protein, an AAV9 VP2 capsid protein, or an AAV9 VP3 capsid protein,
- (ii) the SV40 capsid protein is a SV40 VP1 capsid protein, a SV40 VP2 capsid protein, or a SV40 VP3 capsid protein,
- (iii) the BKV capsid protein is a BKV VP1 capsid protein, a BKV VP2 capsid protein, or a BKV VP3 capsid protein,
- (iv) the JCV capsid protein is a JCV VP1 capsid protein, a JCV VP2 capsid protein, or a JCV VP3 capsid protein, or
- (v) the MPyV capsid protein is a MPyV VP1 capsid protein, a MPyV VP2 capsid protein, or a MPyV VP3 capsid protein.

In still one even more preferred embodiment,
the SV40 VP1 capsid protein has an amino acid sequence according to SEQ ID NO: 1 or an amino acid sequence having at least 80%, preferably at least 85%, more preferably at least 90%, and most preferably at least 95% or 99%, i.e. 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99%, sequence identity thereto,
the SV40 VP2 capsid protein has an amino acid sequence according to SEQ ID NO: 2 or an amino acid sequence having at least 80%, preferably at least 85%, more preferably at least 90%, and most preferably at least 95% or 99%, i.e. 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99%, sequence identity thereto,
the SV40 VP2 capsid protein without DNA binding domain (woDBD) has an amino acid sequence according to SEQ ID NO: 3 or an amino acid sequence having at least 80%, preferably at least 85%, more preferably at least 90%, and most preferably at least 95% or 99%, i.e. 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99%, sequence identity thereto
the SV40 VP3 capsid protein has an amino acid sequence according to SEQ ID NO: 4 or an amino acid sequence having at least 80%, preferably at least 85%, more preferably at least 90%, and most preferably at least 95% or 99%, i.e. 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99%, sequence identity thereto,
the nucleocapsid (NC) protein has an amino acid sequence encompassed by amino acid sequence according to SEQ ID NO: 31 or an amino acid sequence having at least 80%, preferably at least 85%, more preferably at least 90%, and most preferably at least 95% or 99%, i.e. 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99%, sequence identity thereto,
the matrix protein (MA) has an amino acid sequence encompassed by amino acid sequence according to SEQ ID NO: 31 or an amino acid sequence having at least 80%, preferably at least 85%, more preferably at least 90%, and most preferably at least 95% or 99%, i.e. 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99%, sequence identity thereto,
or
the capsid protein (CA) has an amino acid sequence encompassed by amino acid sequence according to SEQ ID NO: 31 or an amino acid sequence having at least 80%, preferably at least 85%, more preferably at least 90%, and most preferably at least 95% or 99%, i.e. 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99%, sequence identity thereto.
A capsid protein having at least 80%, preferably at least 85%, more preferably at least 90%, and most preferably at least 95% or 99%, i.e. 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92,

93, 94, 95, 96, 97, 98, or 99%, sequence identity to the amino acid sequence according to SEQ ID NO: 1 to SEQ ID NO: 4 can be designated as a capsid protein variant. Such a capsid protein variant is still able to make up/form the capsid of a virus or is still able to facilitate the make up/formation of the capsid of a virus. The experimental section provides, for example, sufficient information in this respect. The same applies to SEQ ID NO: 31 variants.

In one most preferred embodiment, the polypeptides comprising a capsid protein of a virus and at least one RNA binding domain have an amino acid sequence according to SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, or SEQ ID NO: 24, or have an amino acid sequence encompassed by amino acid sequence according to SEQ ID NO: 33. Variants are also encompassed. Said variants have 80% sequence identity, preferably at least 85%, more preferably at least 90%, and most preferably at least 95% or 99%, i.e. 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99%, sequence identity to the above sequences.

Specific combinations of a capsid protein of a DNA virus or a fragment or a derivative thereof having capsid protein function connected to/fused to/associated with an RNA binding domain are shown in **Figure 3**. For example, the SV40 VP2 capsid protein can be used in combination with the indicated linker and with any one of the RNA binding domains listed in this Figure. For example, the SV40 VP3 capsid protein can be used in combination with the indicated linker and with any one of the RNA binding domains listed in this Figure. For example, the BKV VP2 capsid protein can be used in combination with the indicated linker and with any one of the RNA binding domains listed in this Figure. For example, the BKV VP3 capsid protein can be used in combination with the indicated linker and with any one of the RNA binding domains listed in this Figure. For example, the JCV VP2 capsid protein can be used in combination with the indicated linker and with any one of the RNA binding domains listed in this Figure. For example, the JCV VP3 capsid protein can be used in combination with the indicated linker and with any one of the RNA binding domains listed in this Figure. For example, the MPyV VP2 capsid protein can be used in combination with the indicated linker and with any one of the RNA binding domains listed in this Figure. For example, the MPyV VP3 capsid protein can be used in combination with the indicated linker and with any one of the RNA binding domains listed in this Figure. For example, the SV40 VP2 capsid protein without DNA binding domain (woDBD) can be used in combination with the indicated linker and with any one of the RNA binding domains listed in this Figure. For example, the SV40 VP3 capsid protein without DNA binding domain (woDBD) can be used in combination with the indicated linker and with any one of the RNA binding domains listed in this Figure. For example, the BKV VP2 capsid protein without DNA binding domain (woDBD) can be used in

combination with the indicated linker and with any one of the RNA binding domains listed in this Figure. For example, the BKV VP3 capsid protein without DNA binding domain (woDBD) can be used in combination with the indicated linker and with any one of the RNA binding domains listed in this Figure. For example, the JCV VP2 capsid protein without DNA binding domain (woDBD) can be used in combination with the indicated linker and with any one of the RNA binding domains listed in this Figure. For example, the JCV VP3 capsid protein without DNA binding domain (woDBD) can be used in combination with the indicated linker and with any one of the RNA binding domains listed in this Figure. For example, the MPyV VP2 capsid protein without DNA binding domain (woDBD) can be used in combination with the indicated linker and with any one of the RNA binding domains listed in this Figure. For example, the MPyV VP3 capsid protein without DNA binding domain (woDBD) can be used in combination with the indicated linker and with any one of the RNA binding domains listed in this Figure. For example, the SV40 VP1 capsid protein can be used in combination with the indicated linker and with any one of the RNA binding domains listed in this Figure. For example, the BKV VP1 capsid protein can be used in combination with the indicated linker and with any one of the RNA binding domains listed in this Figure. For example, the JCV VP1 capsid protein can be used in combination with the indicated linker and with any one of the RNA binding domains listed in this Figure. For example, the MPyV VP1 capsid protein can be used in combination with the indicated linker and with any one of the RNA binding domains listed in this Figure. For example, the AAV VP1 capsid protein can be used in combination with the indicated linker and with any one of the RNA binding domains listed in this Figure. For example, the AAV VP2 capsid protein can be used in combination with the indicated linker and with any one of the RNA binding domains listed in this Figure. For example, the AAV VP3 capsid protein can be used in combination with the indicated linker and with any one of the RNA binding domains listed in this Figure.

In a fifth aspect, the present invention relates to a virus like particle (VLP) which comprises the complex according to the fourth aspect.

The virus like particle (VLP) may be a DNA or RNA virus like particle (VLP).

In one embodiment,

the DNA virus like particle is selected from the group consisting of a papovavirus like particle, a papilloma virus like particle, a polyoma virus like particle, an adenovirus like particle, and a parvovirus virus like particle, or

the RNA virus like particle is a retrovirus like particle, a norovirus like particle, or an orthomyxovirus like particle.

In one preferred embodiment,
the polyoma virus like particle is a Simian Virus 40 (SV40) like particle, a BK virus (BKV) like particle, a JC virus (JCV) like particle, or a MPy virus (MPyV) like particle,
the parvovirus like particle is an adeno-associated virus (AAV) like particle,
the retrovirus like particle is a lentivirus like particle,
the norovirus like particle is a Norwalk virus like particle, or
the orthomyxovirus like particle is an influenza A or influenza B virus like particle.

It is mentioned in the fourth aspect of the present invention that the polypeptide, as component of the complex, comprises a capsid protein of a virus or a fragment or a derivative thereof having capsid protein function and at least one RNA binding domain. The (artificial) circular RNA molecule, as additional component of the complex, preferably comprises an RNA element of interest and a recruiting RNA motif. The recruiting RNA motif interacts with/binds to the at least one RNA binding domain. In this way, the (artificial) circular RNA molecule (comprising the RNA element of interest) is encapsidated/encapsulated in the virus like particle (VLP).

In one particular embodiment, the virus like particle is a SV40 virus like particle comprising, as part of the complex, a polypeptide comprising a SV40 VP1 capsid protein or a fragment or a derivative thereof and at least one RNA binding domain.

In one another particular embodiment, the virus like particle is a SV40 virus like particle comprising, as part of the complex, a polypeptide comprising a SV40 VP2 capsid protein or a fragment or a derivative thereof and at least one RNA binding domain. The SV40 virus like particle comprising a polypeptide comprising a SV40 VP2 capsid protein or a fragment or a derivative thereof and at least one RNA binding domain preferably further comprises the SV40 capsid protein VP1 or the SV40 capsid proteins VP1 and VP3.

In one another particular embodiment, the virus like particle is a SV40 virus like particle, comprising as part of the complex, a polypeptide comprising a SV40 VP3 capsid protein or a fragment or a derivative thereof and at least one RNA binding domain. The SV40 virus like particle comprising a polypeptide comprising SV40 VP3 capsid protein or a fragment or a derivative thereof and at least one RNA binding domain preferably further comprises the SV40 capsid protein VP1 or the SV40 capsid proteins VP1 and VP2.

In one alternative embodiment, the virus like particle is an AAV virus like particle, comprising as part of the complex, a polypeptide comprising an AAV VP3 capsid protein or a fragment or a derivative thereof and at least one RNA binding domain.

In one another alternative embodiment, the virus like particle is an AAV virus like particle, comprising as part of the complex, a polypeptide comprising an AAV VP2 capsid protein or a fragment or a derivative thereof and at least one RNA binding domain. The AAV virus like particle comprising a polypeptide comprising an AAV VP2 capsid protein or a fragment or a derivative thereof and at least one RNA binding domain preferably further comprises the AAV capsid protein VP3 or the AAV capsid proteins VP3 and VP1.

In one another alternative embodiment, the virus like particle is an AAV virus like particle, comprising as part of the complex, a polypeptide comprising an AAV VP1 capsid protein or a fragment or a derivative thereof and at least one RNA binding domain. The AAV virus like particle comprising a polypeptide comprising an AAV VP1 capsid protein or a fragment or a derivative thereof and at least one RNA binding domain preferably further comprises the AAV capsid protein VP3 or the AAV capsid proteins VP3 and VP2.

In one alternative embodiment, the virus like particle is a lentivirus like particle, comprising as part of the complex, a polypeptide comprising a nucleocapsid (NC) protein or a fragment or a derivative thereof and at least one RNA binding domain. The lentivirus like particle comprising a polypeptide comprising a nucleocapsid (NC) protein or a fragment or a derivative thereof and at least one RNA binding domain preferably further comprises the lentivirus matrix protein (MA) and/or the capsid protein (CA).

In one another alternative embodiment, the virus like particle is a lentivirus like particle, comprising as part of the complex, a polypeptide comprising a matrix protein (MA) or a fragment or a derivative thereof and at least one RNA binding domain. The lentivirus like particle comprising a polypeptide comprising a matrix protein (MA) or a fragment or a derivative thereof and at least one RNA binding domain preferably further comprises the lentivirus nucleocapsid (NC) protein and/or the capsid protein (CA).

In one another alternative embodiment, the virus like particle is a lentivirus like particle, comprising as part of the complex, a polypeptide comprising a capsid protein (CA) or a fragment or a derivative thereof and at least one RNA binding domain. The lentivirus like particle comprising a polypeptide comprising a capsid protein (CA) or a fragment or a derivative thereof and at least one RNA binding domain preferably further comprises the lentivirus nucleocapsid (NC) protein and/or the matrix protein (MA).

In the above described particular and alternative embodiments, it is further preferred that

- (i) the at least one RNA binding domain is a MS2 coat protein or an RNA-binding section thereof and the recruiting RNA motif is a MS2 phage operator stem-loop,

- (ii) the at least one RNA binding domain is a Ku protein or an RNA-binding section thereof and the recruiting RNA motif is a telomerase Ku binding motif,
- (iii) the at least one RNA binding domain is a Sm7 protein or an RNA-binding section thereof and the recruiting RNA motif is a telomerase Sm7 binding motif,
- (iv) the at least one RNA binding domain is a SfMu phage COM RNA binding protein or an RNA-binding section thereof and the recruiting RNA motif is a SfMu phage COM stem-loop, or
- (v) the at least one RNA binding domain is a PP7 coat protein or an RNA-binding section thereof and the recruiting RNA motif is PP7 phage operator stem-loop,

and/or

it is further preferred that

the RNA element of interest is selected from the group consisting of a shRNA, miRNA, protein encoding RNA, pre-miRNA, piRNA, RNA complementary to other non-coding RNA, and a ribozyme.

In a sixth aspect, the present invention relates to a virus like particle (VLP) which comprises a (an artificial) circular RNA molecule comprising an RNA element of interest.

Specifically, the circular RNA molecule is an artificial circular RNA molecule, preferably the artificial circular RNA molecule according to the third aspect.

Especially, the (artificial) circular RNA molecule comprised in the virus like particle (VLP) is encapsidated/encapsulated in the VLP.

Particularly, the (encapsidated/encapsulated) (artificial) circular RNA molecule further comprises a recruiting RNA motif. Thus, more particularly, the (encapsidated/encapsulated) (artificial) circular RNA molecule comprises an RNA element of interest and a recruiting RNA motif. The RNA element of interest has a specific function or is of a specific purpose, and is, therefore, of interest. The function of the RNA element of interest is, for example, the inhibition of the expression of genes, e.g. by application of antisense RNA. Alternatively, therapeutic active RNA may be applied to induce gene expression, e.g. to elicit an immune response. The RNA element of interest may be a coding or a non-coding RNA element.

Preferably, the RNA element of interest is selected from the group consisting of a shRNA, miRNA, protein encoding RNA, pre-miRNA, piRNA, RNA complementary to other non-coding RNA, and a ribozyme.

Preferably, the recruiting RNA motif is selected from the group consisting of a MS2 phage operator stem-loop, a telomerase Ku binding motif, a telomerase Sm7 binding motif, a SfMu phage COM stem-loop, and a PP7 phage operator stem-loop.

More preferably, the RNA molecule comprises an RNA element of interest selected from the group consisting of a shRNA, miRNA, protein encoding RNA, pre-miRNA, piRNA, RNA complementary to other non-coding RNA, and a ribozyme and a recruiting RNA motif selected from the group consisting of a MS2 phage operator stem-loop, a telomerase Ku binding motif, a telomerase Sm7 binding motif, a SfMu phage COM stem-loop, and a PP7 phage operator stem-loop.

More specifically, the (encapsidated/encapsulated) (artificial) circular RNA molecule binds to at least one RNA binding domain being part of a polypeptide further comprising a capsid protein of a virus or a fragment or a derivative thereof having capsid protein function via the recruiting RNA motif comprised therein. Even more specifically, the (encapsidated/encapsulated) (artificial) circular RNA molecule binds to at least one RNA binding domain connected to/fused to/associated with a capsid protein of a virus or a fragment or a derivative thereof having capsid protein function via the recruiting RNA motif comprised therein. The polypeptide comprising a capsid protein of a virus or a fragment or a derivative thereof having capsid protein function and at least one RNA binding domain is part of/comprised in the virus like particle (VLP) as described above.

In one preferred embodiment,

- (i) the at least one RNA binding domain is a MS2 coat protein or an RNA-binding section thereof and the recruiting RNA motif is a MS2 phage operator stem-loop,
- (ii) the at least one RNA binding domain is a Ku protein or an RNA-binding section thereof and the recruiting RNA motif is a telomerase Ku binding motif,
- (iii) the at least one RNA binding domain is a Sm7 protein or an RNA-binding section thereof and the recruiting RNA motif is a telomerase Sm7 binding motif,
- (iv) the at least one RNA binding domain is a SfMu phage COM RNA binding protein or an RNA-binding section thereof and the recruiting RNA motif is a SfMu phage COM stem-loop, and/or
- (v) the at least one RNA binding domain is a PP7 coat protein or an RNA-binding section thereof and the recruiting RNA motif is PP7 phage operator stem-loop.

In one more preferred embodiment,

the at least one RNA binding domain has an amino acid sequence selected from the group consisting of SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, and an amino acid sequence having at least 80%, preferably at least 85%, more preferably at least 90%, and most preferably at least 95% or 99%, i.e. 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99%, sequence identity thereto, and/or

the recruiting RNA motif has a nucleotide sequence selected from the group consisting of SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15 (or is a nucleotide sequence transcribed thereof), and a nucleotide sequence having at least 80%, preferably at least 85%, more preferably at least 90%, and most preferably at least 95% or 99%, i.e. 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99%, sequence identity thereto.

The virus like particle (VLP) may be a DNA or RNA virus like particle (VLP).

In one embodiment,

the DNA virus like particle is selected from the group consisting of a papovavirus like particle, a papilloma virus like particle, a polyoma virus like particle, an adenovirus like particle, and a parvovirus virus like particle, or

the RNA virus like particle is a retrovirus like particle, a norovirus like particle, or an orthomyxovirus like particle.

In one preferred embodiment,

the polyoma virus like particle is a Simian Virus 40 (SV40) like particle, a BK virus (BKV) like particle, a JC virus (JCV) like particle, or a MPy virus (MPyV) like particle,

the parvovirus like particle is an adeno-associated virus (AAV) like particle,

the retrovirus like particle is a lentivirus like particle,

the norovirus like particle is a Norwalk virus like particle, or

the orthomyxovirus like particle is an influenza A or influenza B virus like particle.

The capsid protein may be a capsid protein of an RNA or a DNA virus.

In one another embodiment,

the capsid protein of a DNA virus is selected from the group consisting of a papovavirus capsid protein, a papilloma virus capsid protein, a polyoma virus capsid protein, an adenovirus capsid protein, and a parvovirus capsid protein, or

the capsid protein of an RNA virus is a lentivirus capsid protein.

In one preferred embodiment,

- (i) the parvovirus capsid protein is an adeno-associated virus capsid protein, preferably an AAV9 capsid protein,
- (ii) the polyoma virus capsid protein is a SV40 capsid protein, a BKV capsid protein, a JCV capsid protein, or a MPyV capsid protein, or
- (iii) the lentivirus capsid protein is a nucleocapsid (NC) protein, a matrix protein (MA) or a capsid protein (CA).

In one even more preferred embodiment,

- (i) the AAV9 capsid protein is an AAV9 VP1 capsid protein, an AAV9 VP2 capsid protein, or an AAV9 VP3 capsid protein,
- (ii) the SV40 capsid protein is a SV40 VP1 capsid protein, a SV40 VP2 capsid protein, or a SV40 VP3 capsid protein,
- (iii) the BKV capsid protein is a BKV VP1 capsid protein, a BKV VP2 capsid protein, or a BKV VP3 capsid protein,
- (iv) the JCV capsid protein is a JCV VP1 capsid protein, a JCV VP2 capsid protein, or a JCV VP3 capsid protein, or
- (v) the MPyV capsid protein is a MPyV VP1 capsid protein, a MPyV VP2 capsid protein, or a MPyV VP3 capsid protein.

In still one even more preferred embodiment,

the SV40 VP1 capsid protein has an amino acid sequence according to SEQ ID NO: 1 or an amino acid sequence having at least 80%, preferably at least 85%, more preferably at least 90%, and most preferably at least 95% or 99%, i.e. 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99%, sequence identity thereto,

the SV40 VP2 capsid protein has an amino acid sequence according to SEQ ID NO: 2 or an amino acid sequence having at least 80%, preferably at least 85%, more preferably at least 90%, and most preferably at least 95% or 99%, i.e. 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99%, sequence identity thereto,

the SV40 VP2 capsid protein without DNA binding domain (woDBD) has an amino acid sequence according to SEQ ID NO: 3 or an amino acid sequence having at least 80%, preferably at least 85%, more preferably at least 90%, and most preferably at least 95% or 99%, i.e. 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99%, sequence identity thereto

the SV40 VP3 capsid protein has an amino acid sequence according to SEQ ID NO: 4 or an amino acid sequence having at least 80%, preferably at least 85%, more preferably at least 90%, and most preferably at least 95% or 99%, i.e. 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99%, sequence identity thereto,

the nucleocapsid (NC) protein has an amino acid sequence encompassed by amino acid sequence according to SEQ ID NO: 31 or an amino acid sequence having at least 80%, preferably at least 85%, more preferably at least 90%, and most preferably at least 95% or 99%, i.e. 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99%, sequence identity thereto,

the matrix protein (MA) has an amino acid sequence encompassed by amino acid sequence according to SEQ ID NO: 31 or an amino acid sequence having at least 80%, preferably at least 85%, more preferably at least 90%, and most preferably at least 95% or 99%, i.e. 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99%, sequence identity thereto, or

the capsid protein (CA) has an amino acid sequence encompassed by amino acid sequence according to SEQ ID NO: 31 or an amino acid sequence having at least 80%, preferably at least 85%, more preferably at least 90%, and most preferably at least 95% or 99%, i.e. 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99%, sequence identity thereto. A capsid protein having at least 80%, preferably at least 85%, more preferably at least 90%, and most preferably at least 95% or 99%, i.e. 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99%, sequence identity to the amino acid sequence according to SEQ ID NO: 1 to SEQ ID NO: 4 can be designated as a capsid protein variant. Such a capsid protein variant is still able to make up/form the capsid of a virus or is still able to facilitate the make up/formation of the capsid of a virus. The experimental section provides, for example, sufficient information in this respect. The same applies to SEQ ID NO: 31 variants.

In one most preferred embodiment, the polypeptides comprising a capsid protein of a virus and at least one RNA binding domain have an amino acid sequence according to SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, or SEQ ID NO: 24, or have an amino acid sequence encompassed by amino acid sequence according to SEQ ID NO: 33. Variants are also encompassed. Said variants have 80% sequence identity, preferably at least 85%, more preferably at least 90%, and most preferably at least 95% or 99%, i.e. 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99%, sequence identity to the above sequences.

Specific combinations of a capsid protein of a virus or a fragment or a derivative thereof having capsid protein function fused with an RNA binding domain are shown in **Figure 3**.

As mentioned above, the capsid protein of a virus or a fragment or a derivative thereof having capsid protein function and the at least one RNA binding domain are preferably part of a polypeptide. This polypeptide is part of/comprised in the virus like particle (VLP). The (artificial) circular RNA molecule preferably comprises an RNA element of interest and a recruiting RNA motif. The recruiting RNA motif interacts with/binds to the RNA binding domain. In this way, the (artificial) circular RNA molecule (comprising the RNA element of interest) is encapsidated/encapsulated in the virus like particle (VLP).

In one particular embodiment, the virus like particle is a SV40 virus like particle comprising a polypeptide comprising a SV40 VP1 capsid protein or a fragment or a derivative thereof and at least one RNA binding domain.

In one another particular embodiment, the virus like particle is a SV40 virus like particle comprising a polypeptide comprising a SV40 VP2 capsid protein or a fragment or a derivative thereof and at least one RNA binding domain. The SV40 virus like particle comprising a polypeptide comprising a SV40 VP2 capsid protein or a fragment or a derivative thereof and at least one RNA binding domain preferably further comprises the SV40 capsid protein VP1 or the SV40 capsid proteins VP1 and VP3.

In one another particular embodiment, the virus like particle is a SV40 virus like particle comprising a polypeptide comprising a SV40 VP3 capsid protein or a fragment or a derivative thereof and at least one RNA binding domain. The SV40 virus like particle comprising a polypeptide comprising a SV40 VP3 capsid protein or a fragment or a derivative thereof and at least one RNA binding domain preferably further comprises the SV40 capsid protein VP1 or the SV40 capsid proteins VP1 and VP2.

In one alternative embodiment, the virus like particle is an AAV virus like particle comprising a polypeptide comprising an AAV VP3 capsid protein or a fragment or a derivative thereof and at least one RNA binding domain.

In one another alternative embodiment, the virus like particle is an AAV virus like particle comprising a polypeptide comprising an AAV VP2 capsid protein or a fragment or a derivative thereof and at least one RNA binding domain. The AAV virus like particle comprising a polypeptide comprising an AAV VP2 capsid protein or a fragment or a derivative thereof and at least one RNA binding domain preferably further comprises the AAV capsid protein VP3 or the AAV capsid proteins VP3 and VP1.

In one another alternative embodiment, the virus like particle is an AAV virus like particle comprising a polypeptide comprising an AAV VP1 capsid protein or a fragment or a derivative thereof and at least one RNA binding domain. The AAV virus like particle comprising a polypeptide comprising an AAV VP1 capsid protein or a fragment or a derivative thereof and at least one RNA binding domain preferably further comprises the AAV capsid protein VP3 or the AAV capsid proteins VP3 and VP2.

In one alternative embodiment, the virus like particle is a lentivirus like particle comprising a polypeptide comprising a nucleocapsid (NC) protein or a fragment or a derivative thereof and at least one RNA binding domain. The lentivirus like particle comprising a polypeptide comprising a nucleocapsid (NC) protein or a fragment or a derivative thereof and

at least one RNA binding domain preferably further comprises the lentivirus matrix protein (MA) and/or a capsid protein (CA).

In one another alternative embodiment, the virus like particle is a lentivirus like particle comprising a polypeptide comprising a matrix protein (MA) or a fragment or a derivative thereof and at least one RNA binding domain. The lentivirus like particle comprising a polypeptide comprising a matrix protein (MA) or a fragment or a derivative thereof and at least one RNA binding domain preferably further comprises the lentivirus nucleocapsid (NC) protein and/or a capsid protein (CA).

In one another alternative embodiment, the virus like particle is a lentivirus like particle comprising a polypeptide comprising a capsid protein (CA) or a fragment or a derivative thereof and at least one RNA binding domain. The lentivirus like particle comprising a polypeptide comprising a capsid protein (CA) or a fragment or a derivative thereof and at least one RNA binding domain preferably further comprises the lentivirus nucleocapsid (NC) protein and/or a matrix protein (MA).

In the above described particular and alternative embodiments, it is further preferred that

- (i) the at least one RNA binding domain is a MS2 coat protein or an RNA-binding section thereof and the recruiting RNA motif is a MS2 phage operator stem-loop,
- (ii) the at least one RNA binding domain is a Ku protein or an RNA-binding section thereof and the recruiting RNA motif is a telomerase Ku binding motif,
- (iii) the at least one RNA binding domain is a Sm7 protein or an RNA-binding section thereof and the recruiting RNA motif is a telomerase Sm7 binding motif,
- (iv) the at least one RNA binding domain is a SfMu phage COM RNA binding protein or an RNA-binding section thereof and the recruiting RNA motif is a SfMu phage COM stem-loop, or
- (v) the at least one RNA binding domain is a PP7 coat protein or an RNA-binding section thereof and the recruiting RNA motif is PP7 phage operator stem-loop,

and/or

it is further preferred that

the RNA element of interest is selected from the group consisting of a shRNA, miRNA, protein encoding RNA, pre-miRNA, piRNA, RNA complementary to other non-coding RNA, and a ribozyme.

In one alternative embodiment, the virus like particle (VLP) comprises the complex according to the fourth aspect of the present invention.

In a seventh aspect, the present invention relates to a (an *in vitro*) method for producing a virus like particle (VLP) which comprises an artificial circular RNA molecule comprising an RNA element of interest, preferably as defined in the fifth or sixth aspect, comprising the steps of:

- (i) providing a cell, and
- (ii) introducing
 - (iia) a polypeptide comprising a capsid protein of a virus or a fragment or a derivative thereof having capsid protein function and at least one RNA binding domain, a polynucleotide encoding the polypeptide, or a vector comprising the polynucleotide, and
 - (iib) a (linear) polynucleotide (capable of circularization) according to the first aspect, a vector according to the second aspect, an artificial circular RNA molecule comprising an RNA element of interest, or an artificial circular RNA molecule according to the third aspect

into the cell,

thereby producing the virus like particle (VLP) comprising an artificial circular RNA molecule comprising an RNA element of interest.

The introduction of the polypeptide into the cell in step (ii/iia) can take place via microinjection, electroporation, or lipofection. The polynucleotide, preferably integrated in a vector, e.g. an expression vector, can be introduced into the cell in step (ii/iia) via transfection, transformation, microinjection, electroporation, or lipofection. The polynucleotide is subsequently transcribed or transcribed and translated into the respective product within the cell. The person skilled in the art is well informed about molecular biological techniques, such as transfection, transformation, microinjection, electroporation, or lipofection, for introducing polypeptides or polynucleotides into a cell and knows how to perform these techniques.

The introduction of the (linear) polynucleotide (capable of circularization), preferably integrated in a vector into the cell in step (ii/iib) can take place via transfection, transformation, microinjection, electroporation, or lipofection. The artificial circular RNA molecule can be introduced into the cell in step (ii/iib) accordingly.

As mentioned above, a polypeptide comprising a capsid protein of a virus or a fragment or a derivative thereof having capsid protein function and at least one RNA binding domain is introduced into the cell in step (ii/iia).

In one embodiment, the at least one RNA binding domain is selected from the group consisting of a MS2 coat protein or an RNA-binding section thereof, a Ku protein or an RNA-binding

section thereof, a Sm7 protein or an RNA-binding section thereof, a SfMu phage COM RNA binding protein or an RNA-binding section thereof, and a PP7 coat protein or an RNA-binding section thereof.

In one more preferred embodiment, the at least one RNA binding domain has an amino acid sequence selected from the group consisting of SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, and an amino acid sequence having at least 80%, preferably at least 85%, more preferably at least 90%, and most preferably at least 95% or 99%, i.e. 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99%, sequence identity thereto.

The capsid protein may be a capsid protein of an RNA or a DNA virus.

In one another embodiment,

the capsid protein of a DNA virus is selected from the group consisting of a papovavirus capsid protein, a papilloma virus capsid protein, a polyoma virus capsid protein, an adenovirus capsid protein, and a parvovirus capsid protein, or

the capsid protein of an RNA virus is a lentivirus capsid protein.

In one preferred embodiment,

- (i) the parvovirus capsid protein is an adeno-associated virus capsid protein, preferably an AAV9 capsid protein,
- (ii) the polyoma virus capsid protein is a SV40 capsid protein, a BKV capsid protein, a JCV capsid protein, or a MPyV capsid protein, or
- (iii) the lentivirus capsid protein is a nucleocapsid (NC) protein, a matrix protein (MA) or a capsid protein (CA).

In one more preferred embodiment,

- (i) the AAV9 capsid protein is an AAV9 VP1 capsid protein, an AAV9 VP2 capsid protein, or an AAV9 VP3 capsid protein,
- (ii) the SV40 capsid protein is a SV40 VP1 capsid protein, a SV40 VP2 capsid protein, or a SV40 VP3 capsid protein,
- (iii) the BKV capsid protein is a BKV VP1 capsid protein, a BKV VP2 capsid protein, or a BKV VP3 capsid protein,
- (iv) the JCV capsid protein is a JCV VP1 capsid protein, a JCV VP2 capsid protein, or a JCV VP3 capsid protein, or
- (v) the MPyV capsid protein is a MPyV VP1 capsid protein, a MPyV VP2 capsid protein, or a MPyV VP3 capsid protein.

In one even more preferred embodiment,

the SV40 VP1 capsid protein has an amino acid sequence according to SEQ ID NO: 1 or an amino acid sequence having at least 80%, preferably at least 85%, more preferably at least 90%, and most preferably at least 95% or 99%, i.e. 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99%, sequence identity thereto,

the SV40 VP2 capsid protein has an amino acid sequence according to SEQ ID NO: 2 or an amino acid sequence having at least 80%, preferably at least 85%, more preferably at least 90%, and most preferably at least 95% or 99%, i.e. 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99%, sequence identity thereto,

the SV40 VP2 capsid protein without DNA binding domain (woDBD) has an amino acid sequence according to SEQ ID NO: 3 or an amino acid sequence having at least 80%, preferably at least 85%, more preferably at least 90%, and most preferably at least 95% or 99%, i.e. 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99%, sequence identity thereto

the SV40 VP3 capsid protein has an amino acid sequence according to SEQ ID NO: 4 or an amino acid sequence having at least 80%, preferably at least 85%, more preferably at least 90%, and most preferably at least 95% or 99%, i.e. 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99%, sequence identity thereto,

the nucleocapsid (NC) protein has an amino acid sequence encompassed by amino acid sequence according to SEQ ID NO: 31 or an amino acid sequence having at least 80%, preferably at least 85%, more preferably at least 90%, and most preferably at least 95% or 99%, i.e. 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99%, sequence identity thereto,

the matrix protein (MA) has an amino acid sequence encompassed by amino acid sequence according to SEQ ID NO: 31 or an amino acid sequence having at least 80%, preferably at least 85%, more preferably at least 90%, and most preferably at least 95% or 99%, i.e. 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99%, sequence identity thereto, or

the capsid protein (CA) has an amino acid sequence encompassed by amino acid sequence according to SEQ ID NO: 31 or an amino acid sequence having at least 80%, preferably at least 85%, more preferably at least 90%, and most preferably at least 95% or 99%, i.e. 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99%, sequence identity thereto. A capsid protein having at least 80%, preferably at least 85%, more preferably at least 90%, and most preferably at least 95% or 99%, i.e. 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99%, sequence identity to the amino acid sequence according to SEQ

ID NO: 1 to SEQ ID NO: 4 can be designated as a capsid protein variant. Such a capsid protein variant is still able to make up/form the capsid of a virus or is still able to facilitate the make up/formation of the capsid of a virus. The experimental section provides, for example, sufficient information in this respect. The same applies to SEQ ID NO: 31 variants.

In one most preferred embodiment, the polypeptides comprising a capsid protein of a virus and at least one RNA binding domain have an amino acid sequence according to SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, or SEQ ID NO: 24, or have an amino acid sequence encompassed by amino acid sequence according to SEQ ID NO: 33. Variants are also encompassed. Said variants have 80% sequence identity, preferably at least 85%, more preferably at least 90%, and most preferably at least 95% or 99%, i.e. 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99%, sequence identity to the above sequences.

Specific combinations of a capsid protein of a virus or a fragment or a derivative thereof having capsid protein function fused with an RNA binding domain are shown in **Figure 3**.

As mentioned above, an artificial circular RNA molecule is introduced into the cell in step (ii/iib). Particularly, the artificial circular RNA molecule comprises an RNA element of interest. More particularly, the artificial circular RNA molecule further comprises a recruiting RNA motif. Thus, even more particularly, the artificial circular RNA molecule comprises an RNA element of interest and a recruiting RNA motif.

The RNA element of interest has a specific function or is of a specific purpose, and is, therefore, of interest. The function of the RNA element of interest is, for example, the inhibition of the expression of genes, e.g. by application of antisense RNA. Alternatively, therapeutic active RNA may be applied to induce gene expression, e.g. to elicit an immune response. The RNA element of interest may be a coding or a non-coding RNA element.

Preferably, the RNA element of interest is selected from the group consisting of a shRNA, miRNA, protein encoding RNA, pre-miRNA, piRNA, RNA complementary to other non-coding RNA, and a ribozyme.

Preferably, the recruiting RNA motif is selected from the group consisting of a MS2 phage operator stem-loop, a telomerase Ku binding motif, a telomerase Sm7 binding motif, a SfMu phage COM stem-loop, and a PP7 phage operator stem-loop.

More preferably, the recruiting RNA motif has a nucleotide sequence selected from the group consisting of SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15 (or is a nucleotide sequence transcribed thereof), and a nucleotide sequence having at least 80%, preferably at least 85%, more preferably at least 90%, and most preferably at least 95%

or 99%, i.e. 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99%, sequence identity thereto.

Even more preferably, the artificial circular RNA molecule comprises an RNA element of interest selected from the group consisting of a shRNA, miRNA, protein encoding RNA, pre-miRNA, piRNA, RNA complementary to other non-coding RNA, and a ribozyme and the recruiting RNA motif is selected from the group consisting of a MS2 phage operator stem-loop, a telomerase Ku binding motif, a telomerase Sm7 binding motif, a SfMu phage COM stem-loop, and a PP7 phage operator stem-loop.

Still even more preferably, the artificial circular RNA molecule comprises an RNA element of interest selected from the group consisting of a shRNA, miRNA, protein encoding RNA, pre-miRNA, piRNA, RNA complementary to other non-coding RNA, and a ribozyme and the recruiting RNA motif has a nucleotide sequence selected from the group consisting of SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15 (or is a nucleotide sequence transcribed thereof), and a nucleotide sequence having at least 80%, preferably at least 85%, more preferably at least 90%, and most preferably at least 95% or 99%, i.e. 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99%, sequence identity thereto.

Specifically, the RNA binding domain and the recruiting RNA motif can be a pair selected from the above groups.

As to further preferred embodiments of the artificial circular RNA molecule and the RNA binding domain, it is referred to the third to sixth aspect of the present invention.

As to further preferred embodiments of the virus like particle (VLP), it is referred to the fifth and sixth aspect of the present invention.

In the process of virus like particle (VLP) formation within the cell, the recruiting RNA motif specifically comprised in the artificial circular RNA molecule binds to the at least one RNA binding domain connected to/fused to/associated with a capsid protein of a virus or a fragment or a derivative thereof having capsid protein function. In this way, the artificial circular RNA molecule (and the RNA element of interest further comprised therein) is encapsidated/encapsulated in the capsid structure of the virus like particle (VLP).

In one example, a virus like particle (VLP) of a SV40 virus comprising an artificial circular RNA molecule is produced. For this purpose, a polynucleotide comprising a nucleotide sequence encoding a polypeptide comprising a SV40 VP1 capsid protein or a fragment or a derivative thereof and at least one RNA binding domain is introduced into the cell. The artificial circular RNA molecule introduced into the cell may be an RNA molecule as defined above.

In one another example, a virus like particle (VLP) of an AAV virus comprising an artificial circular RNA molecule is produced. For this purpose, a polynucleotide comprising a nucleotide sequence encoding a polypeptide comprising an AAV VP3 capsid protein or a fragment or a derivative thereof and at least one RNA binding domain is introduced into the cell. The artificial circular RNA molecule introduced into the cell may be an RNA molecule as defined above.

Additional (helper) elements may further be introduced into the cell which assist/facilitate virus like particle (VLP) formation.

In one example, a virus like particle (VLP) of a SV40 virus comprising an artificial circular RNA molecule is produced.

In this process, in addition to a polynucleotide comprising a nucleotide sequence encoding a polypeptide comprising a SV40 VP2 capsid protein or a fragment or a derivative thereof and at least one RNA binding domain,

a polynucleotide comprising a nucleotide sequence encoding a SV40 capsid protein VP1, or a polynucleotide comprising a nucleotide sequence encoding a SV40 capsid protein VP1 and a polynucleotide comprising a nucleotide sequence encoding a SV40 capsid protein VP3 is (are) introduced into the cell.

Alternatively, in addition to a polynucleotide comprising a nucleotide sequence encoding a polypeptide comprising a SV40 VP3 capsid protein or a fragment or a derivative thereof and at least one RNA binding domain,

a polynucleotide comprising a nucleotide sequence encoding a SV40 capsid protein VP1, or a polynucleotide comprising a nucleotide sequence encoding a SV40 capsid protein VP1 and a polynucleotide comprising a nucleotide sequence encoding a SV40 capsid protein VP2 is (are) introduced into the cell.

The above polynucleotides may be comprised in a vector such as viral or plasmid vector. The nucleotide sequences encoding the above capsid proteins may also be part of a single polynucleotide, e.g. comprised in a single vector. For example, the single polynucleotide, e.g. comprised in a single vector, may comprise a nucleotide sequence encoding a polypeptide comprising a SV40 VP3 capsid protein or a fragment or a derivative thereof and at least one RNA binding domain as well as a nucleotide sequence encoding a SV40 VP1 capsid protein and/or a nucleotide sequence encoding a SV40 VP2 capsid protein.

In one another example, a virus like particle (VLP) of an AAV virus comprising an artificial circular RNA molecule is produced.

In this process, in addition to a polynucleotide comprising a nucleotide sequence encoding a polypeptide comprising an AAV VP2 capsid protein or a fragment or a derivative thereof and at least one RNA binding domain,

a polynucleotide comprising a nucleotide sequence encoding an AAV capsid protein VP3, or a polynucleotide comprising a nucleotide sequence encoding an AAV capsid protein VP3 and a polynucleotide comprising a nucleotide sequence encoding an AAV capsid protein VP1 is (are) introduced into the cell.

Alternatively, in addition to a polynucleotide comprising a nucleotide sequence encoding a polypeptide comprising an AAV VP1 capsid protein or a fragment or a derivative thereof and at least one RNA binding domain,

a polynucleotide comprising a nucleotide sequence encoding an AAV capsid protein VP3, or a polynucleotide comprising a nucleotide sequence encoding an AAV capsid protein VP3 and a polynucleotide comprising a nucleotide sequence encoding an AAV capsid protein VP2 is (are) introduced into the cell.

The above polynucleotides may be comprised in a vector such as viral or plasmid vector. The nucleotide sequences encoding the above capsid proteins may also be part of a single polynucleotide, e.g. comprised in a single vector. For example, the single polynucleotide, e.g. comprised in a single vector, may comprise a nucleotide sequence encoding a polypeptide comprising an AAV VP3 capsid protein or a fragment or a derivative thereof and at least one RNA binding domain as well as a nucleotide sequence encoding an AAV VP2 capsid protein and/or a nucleotide sequence encoding an AAV VP1 capsid protein.

When a virus like particle (VLP) of an AAV virus comprising a (heterologous) RNA molecule is produced, it is preferred that additional helper elements are present such as adenovirus helper genes E4, E2a and VA. Said helper elements further assist/facilitate virus like particle (VLP) formation.

In one further example, a virus like particle (VLP) of lentivirus comprising an artificial circular RNA molecule is produced. For this purpose, a polynucleotide comprising a nucleotide sequence encoding a polypeptide comprising a nucleocapsid (NC) protein or a fragment or a derivative thereof and at least one RNA binding domain is introduced into the cell. The artificial circular RNA molecule introduced into the cell may be an RNA molecule as defined above.

Additional (helper) elements may further be introduced into the cell which assist/facilitate virus like particle (VLP) formation.

In one example, a virus like particle (VLP) of a lentivirus comprising an artificial circular RNA molecule is produced.

In this process, in addition to a polynucleotide comprising a nucleotide sequence encoding a polypeptide comprising a nucleocapsid (NC) protein or a fragment or a derivative thereof and at least one RNA binding domain,

a polynucleotide comprising a nucleotide sequence encoding a matrix protein (MA) and/or a polynucleotide comprising a nucleotide sequence encoding a capsid protein (CA) is (are) introduced into the cell.

Alternatively, in addition to a polynucleotide comprising a nucleotide sequence encoding a polypeptide comprising a matrix protein (MA) protein or a fragment or a derivative thereof and at least one RNA binding domain,

a polynucleotide comprising a nucleotide sequence encoding a nucleocapsid (NC) protein and/or a polynucleotide comprising a nucleotide sequence encoding a capsid protein (CA) is (are) introduced into the cell.

Alternatively, in addition to a polynucleotide comprising a nucleotide sequence encoding a polypeptide comprising a capsid protein (CA) or a fragment or a derivative thereof and at least one RNA binding domain,

a polynucleotide comprising a nucleotide sequence encoding a nucleocapsid (NC) protein and/or a polynucleotide comprising a nucleotide sequence encoding a matrix protein (MA) is (are) introduced into the cell.

These additional capsid proteins assist/facilitate VLP formation.

Additionally, the POL gene encoding the viral protease (PRO), the reverse transcriptase (RT) with a non-functional integrase gene (IN) may be introduced into the cell. Moreover, the VSVG (ENV) gene encoding the viral surface glycoprotein (gp160) which is finally splitted into surface protein gp120 (SU) and transmembrane protein gp41 (TM) may be introduced into the cell.

The above polynucleotides may be comprised in a vector such as viral or plasmid vector. The nucleotide sequences encoding the above capsid proteins may also be part of a single polynucleotide, e.g. comprised in a single vector. For example, the single polynucleotide, e.g. comprised in a single vector, may comprise a nucleotide sequence encoding a nucleocapsid (NC) protein and at least one RNA binding domain as well as a nucleotide sequence encoding a matrix protein (MA) and/or a nucleotide sequence encoding a capsid protein (CA).]

In an eighth aspect, the present invention relates to a (an *in vitro*) method for producing a virus like particle (VLP) which comprises an artificial circular RNA molecule comprising an RNA element of interest, preferably as defined in the fifth or sixth aspect, comprising the steps of:

- (i) providing a virus like particle (VLP) which comprises a polypeptide comprising a capsid protein of a virus or a fragment or a derivative thereof having capsid protein function and at least one RNA binding domain,
- (ii) dis-assembling the VLP,
- (iii) adding an artificial circular RNA molecule comprising an RNA element of interest or an artificial circular RNA molecule according to the third aspect,
- (iv) re-assembling the dis-assembled VLP,
thereby producing the virus like particle (VLP) comprising an artificial circular RNA molecule comprising an RNA element of interest.

The virus like particle (VLP) provided in step (i) may be a DNA or RNA virus like particle (VLP).

In one embodiment,

the DNA virus like particle is selected from the group consisting of a papovavirus like particle, a papilloma virus like particle, a polyoma virus like particle, an adenovirus like particle, and a parvovirus virus like particle, or

the RNA virus like particle is a retrovirus like particle, a norovirus like particle, or a orthomyxovirus like particle.

In one preferred embodiment,

the polyoma virus like particle is a Simian Virus 40 (SV40) like particle, a BK virus (BKV) like particle, a JC virus (JCV) like particle, or a MPy virus (MPyV) like particle,

the parvovirus like particle is an adeno-associated virus (AAV) like particle,

the retrovirus like particle is a lentivirus like particle,

the norovirus like particle is a Norwalk virus like particle, or

the orthomyxovirus like particle is an influenza A or influenza B virus like particle.

The capsid protein may be a capsid protein of an RNA or a DNA virus.

In one another embodiment,

the capsid protein of a DNA virus is selected from the group consisting of a papovavirus capsid protein, a papilloma virus capsid protein, a polyoma virus capsid protein, an adenovirus capsid protein, and a parvovirus capsid protein, or

the capsid protein of an RNA virus is a lentivirus capsid protein.

In one preferred embodiment,

- (i) the parvovirus capsid protein is an adeno-associated virus capsid protein, preferably an AAV9 capsid protein,

- (ii) the polyoma virus capsid protein is a SV40 capsid protein, a BKV capsid protein, a JCV capsid protein, or a MPyV capsid protein, or
- (iii) the lentivirus capsid protein is a nucleocapsid (NC) protein, a matrix protein (MA) or a capsid protein (CA).

In one even more preferred embodiment,

- (i) the AAV9 capsid protein is an AAV9 VP1 capsid protein, an AAV9 VP2 capsid protein, or an AAV9 VP3 capsid protein,
- (ii) the SV40 capsid protein is a SV40 VP1 capsid protein, a SV40 VP2 capsid protein, or a SV40 VP3 capsid protein,
- (iii) the BKV capsid protein is a BKV VP1 capsid protein, a BKV VP2 capsid protein, or a BKV VP3 capsid protein,
- (iv) the JCV capsid protein is a JCV VP1 capsid protein, a JCV VP2 capsid protein, or a JCV VP3 capsid protein, or
- (v) the MPyV capsid protein is a MPyV VP1 capsid protein, a MPyV VP2 capsid protein, or a MPyV VP3 capsid protein.

In still one even more preferred embodiment,

the SV40 VP1 capsid protein has an amino acid sequence according to SEQ ID NO: 1 or an amino acid sequence having at least 80%, preferably at least 85%, more preferably at least 90%, and most preferably at least 95% or 99%, i.e. 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99%, sequence identity thereto,

the SV40 VP2 capsid protein has an amino acid sequence according to SEQ ID NO: 2 or an amino acid sequence having at least 80%, preferably at least 85%, more preferably at least 90%, and most preferably at least 95% or 99%, i.e. 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99%, sequence identity thereto,

the SV40 VP2 capsid protein without DNA binding domain (woDBD) has an amino acid sequence according to SEQ ID NO: 3 or an amino acid sequence having at least 80%, preferably at least 85%, more preferably at least 90%, and most preferably at least 95% or 99%, i.e. 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99%, sequence identity thereto

the SV40 VP3 capsid protein has an amino acid sequence according to SEQ ID NO: 4 or an amino acid sequence having at least 80%, preferably at least 85%, more preferably at least 90%, and most preferably at least 95% or 99%, i.e. 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99%, sequence identity thereto,

the nucleocapsid (NC) protein has an amino acid sequence encompassed by amino acid sequence according to SEQ ID NO: 31 or an amino acid sequence having at least 80%, preferably at least 85%, more preferably at least 90%, and most preferably at least 95% or 99%, i.e. 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99%, sequence identity thereto,

the matrix protein (MA) has an amino acid sequence encompassed by amino acid sequence according to SEQ ID NO: 31 or an amino acid sequence having at least 80%, preferably at least 85%, more preferably at least 90%, and most preferably at least 95% or 99%, i.e. 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99%, sequence identity thereto, or

the capsid protein (CA) has an amino acid sequence encompassed by amino acid sequence according to SEQ ID NO: 31 or an amino acid sequence having at least 80%, preferably at least 85%, more preferably at least 90%, and most preferably at least 95% or 99%, i.e. 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99%, sequence identity thereto. A capsid protein having at least 80%, preferably at least 85%, more preferably at least 90%, and most preferably at least 95% or 99%, i.e. 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99%, sequence identity to the amino acid sequence according to SEQ ID NO: 1 to SEQ ID NO: 4 can be designated as a capsid protein variant. Such a capsid protein variant is still able to make up/form the capsid of a virus or is still able to facilitate the make up/formation of the capsid of a virus. The experimental section provides, for example, sufficient information in this respect. The same applies to SEQ ID NO: 31 variants.

In one further embodiment, the at least one RNA binding domain is selected from the group consisting of a MS2 coat protein or an RNA-binding section thereof, a Ku protein or an RNA-binding section thereof, a Sm7 protein or an RNA-binding section thereof, a SfMu phage COM RNA binding protein or an RNA-binding section thereof, and a PP7 coat protein or an RNA-binding section thereof.

In one preferred embodiment, the at least one RNA binding domain has an amino acid sequence selected from the group consisting of SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, and an amino acid sequence having at least 80%, preferably at least 85%, more preferably at least 90%, and most preferably at least 95% or 99%, i.e. 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99%, sequence identity thereto.

In one most preferred embodiment, the polypeptides comprising a capsid protein of a virus and at least one RNA binding domain have an amino acid sequence according to SEQ ID

NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, or SEQ ID NO: 24, or have an amino acid sequence encompassed by amino acid sequence according to SEQ ID NO: 33. Variants are also encompassed. Said variants have 80% sequence identity, preferably at least 85%, more preferably at least 90%, and most preferably at least 95% or 99%, i.e. 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99%, sequence identity to the above sequences.

Specific combinations of a capsid protein of a virus or a fragment or a derivative thereof having capsid protein function fused with an RNA binding domain are shown in **Figure 3**.

In step (iii), an artificial circular RNA molecule is added. Particularly, the artificial circular RNA molecule comprises an RNA element of interest. More particularly, the artificial circular RNA molecule further comprises a recruiting RNA motif. Thus, even more particularly, the artificial circular RNA molecule comprises an RNA element of interest and a recruiting RNA motif.

The RNA element of interest has a specific function or is of a specific purpose, and is, therefore, of interest. The function of the RNA element of interest is, for example, the inhibition of the expression of genes, e.g. by application of antisense RNA. Alternatively, therapeutic active RNA may be applied to induce gene expression, e.g. to elicit an immune response. The RNA element of interest may be a coding or a non-coding RNA element.

Preferably, the RNA element of interest is selected from the group consisting of a shRNA, miRNA, protein encoding RNA, pre-miRNA, piRNA, RNA complementary to other non-coding RNA, and a ribozyme.

Preferably, the recruiting RNA motif is selected from the group consisting of a MS2 phage operator stem-loop, a telomerase Ku binding motif, a telomerase Sm7 binding motif, a SfMu phage COM stem-loop, and a PP7 phage operator stem-loop.

More preferably, the recruiting RNA motif has a nucleotide sequence selected from the group consisting of SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15 (or is a nucleotide sequence transcribed thereof), and a nucleotide sequence having at least 80%, preferably at least 85%, more preferably at least 90%, and most preferably at least 95% or 99%, i.e. 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99%, sequence identity thereto.

Even more preferably, the artificial circular RNA molecule comprises an RNA element of interest selected from the group consisting of a shRNA, miRNA, protein encoding RNA, pre-miRNA, piRNA, RNA complementary to other non-coding RNA, and a ribozyme and the recruiting RNA motif is selected from the group consisting of a MS2 phage operator stem-loop,

a telomerase Ku binding motif, a telomerase Sm7 binding motif, a SfMu phage COM stem-loop, and a PP7 phage operator stem-loop.

Still even more preferably, the artificial circular RNA molecule comprises an RNA element of interest selected from the group consisting of a shRNA, miRNA, protein encoding RNA, pre-miRNA, piRNA, RNA complementary to other non-coding RNA, and a ribozyme and the recruiting RNA motif has a nucleotide sequence selected from the group consisting of SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15 (or is a nucleotide sequence transcribed thereof), and a nucleotide sequence having at least 80%, preferably at least 85%, more preferably at least 90%, and most preferably at least 95% or 99%, i.e. 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99%, sequence identity thereto.

As to further preferred embodiments of the virus like particle provided in step (i), it is referred to the fifth and sixth aspect of the present invention.

As to further preferred embodiments of the artificial circular RNA molecule added in step (iii), it is referred to the third to seventh aspect of the present invention.

In one particular embodiment, the virus like particle provided in step (i) is a SV40 virus like particle comprising a polypeptide comprising a SV40 VP1 capsid protein or a fragment or a derivative thereof and at least one RNA binding domain.

In one another particular embodiment, the virus like particle provided in step (i) is a SV40 virus like particle comprising a polypeptide comprising a SV40 VP2 capsid protein or a fragment or a derivative thereof and at least one RNA binding domain. The SV40 virus like particle comprising a polypeptide comprising a SV40 VP2 capsid protein or a fragment or a derivative thereof and at least one RNA binding domain preferably further comprises the SV40 capsid protein VP1 or the SV40 capsid proteins VP1 and VP3.

In one another particular embodiment, the virus like particle provided in step (i) is a SV40 virus like particle comprising a polypeptide comprising a SV40 VP3 capsid protein or a fragment or a derivative thereof and at least one RNA binding domain. The SV40 virus like particle comprising a polypeptide comprising a SV40 VP3 capsid protein or a fragment or a derivative thereof and at least one RNA binding domain preferably further comprises the SV40 capsid protein VP1 or the SV40 capsid proteins VP1 and VP2.

In the above described particular embodiments, it is further preferred that

- (i) the at least one RNA binding domain comprised in the polypeptide being part of the VLP is a MS2 coat protein or an RNA-binding section thereof and the recruiting RNA

- motif comprised in the artificial circular RNA molecule added is a MS2 phage operator stem-loop,
- (ii) the at least one RNA binding domain comprised in the polypeptide being part of the VLP is a Ku protein or an RNA-binding section thereof and the recruiting RNA motif comprised in the artificial circular RNA molecule added is a telomerase Ku binding motif,
 - (iii) the at least one RNA binding domain comprised in the polypeptide being part of the VLP is a Sm7 protein or an RNA-binding section thereof and the recruiting RNA motif comprised in the artificial circular RNA molecule added is a telomerase Sm7 binding motif,
 - (iv) the at least one RNA binding domain comprised in the polypeptide being part of the VLP is a SfMu phage COM RNA binding protein or an RNA-binding section thereof and the recruiting RNA motif comprised in the artificial circular RNA molecule added is a SfMu phage COM stem-loop, or
 - (v) the at least one RNA binding domain comprised in the polypeptide being part of the VLP is a PP7 coat protein or an RNA-binding section thereof and the recruiting RNA motif comprised in the artificial circular RNA molecule added is PP7 phage operator stem-loop,

and/or

it is further preferred that

the RNA element of interest comprised in the artificial circular RNA molecule added is selected from the group consisting of a shRNA, miRNA, protein encoding RNA, pre-miRNA, piRNA, RNA complementary to other non-coding RNA, and a ribozyme

In one preferred embodiment, the virus like particle (VLP) which comprises a polypeptide comprising a capsid protein of a virus or a fragment or a derivative thereof having capsid protein function and at least one RNA binding domain provided in (i) is produced by

- (a) providing a cell, and
- (b) introducing a polypeptide comprising a capsid protein of a virus or a fragment or a derivative thereof having capsid protein function and at least one RNA binding domain, a polynucleotide encoding the polypeptide, or a vector comprising the polynucleotide into the cell.

The introduction of the polypeptide into the cell in step (b) can take place via microinjection, electroporation, or lipofection. The polynucleotide, preferably integrated in a vector, e.g. an expression vector, can be introduced into the cell in step (b) via transfection,

transformation, microinjection, electroporation, or lipofection. The polynucleotide is subsequently transcribed or transcribed and translated into the respective product within the cell. The person skilled in the art is well informed about molecular biological techniques, such as transfection, transformation, microinjection, electroporation, or lipofection, for introducing polypeptides or polynucleotides into a cell and knows how to perform these techniques.

In one example, a virus like particle (VLP) of a SV40 virus comprising an artificial circular RNA molecule is produced. For this purpose, a polynucleotide comprising a nucleotide sequence encoding a polypeptide comprising a SV40 VP1 capsid protein or a fragment or a derivative thereof and at least one RNA binding domain is introduced into the cell.

Additional elements may further be introduced into the cell which assist/facilitate VLP formation.

In one example, a virus like particle (VLP) of a SV40 virus is produced.

In this process, in addition to a polynucleotide comprising a nucleotide sequence encoding a polypeptide comprising a SV40 VP2 capsid protein or a fragment or a derivative thereof and at least one RNA binding domain,

a polynucleotide comprising a nucleotide sequence encoding a SV40 capsid protein VP1, or a polynucleotide comprising a nucleotide sequence encoding a SV40 capsid protein VP1 and a polynucleotide comprising a nucleotide sequence encoding a SV40 capsid protein VP3 is (are) introduced into the cell.

Alternatively, in addition to a polynucleotide comprising a nucleotide sequence encoding a polypeptide comprising a SV40 VP3 capsid protein or a fragment or a derivative thereof and at least one RNA binding domain,

a polynucleotide comprising a nucleotide sequence encoding a SV40 capsid protein VP1, or a polynucleotide comprising a nucleotide sequence encoding a SV40 capsid protein VP1 and a polynucleotide comprising a nucleotide sequence encoding a SV40 capsid protein VP2 is (are) introduced into the cell.

The above polynucleotides may be comprised in a vector such as viral or plasmid vector. The nucleotide sequences encoding the above capsid proteins may also be part of a single polynucleotide, e.g. comprised in a single vector. For example, the single polynucleotide, e.g. comprised in a single vector, may comprise a nucleotide sequence encoding a polypeptide comprising a SV40 VP3 capsid protein or a fragment or a derivative thereof and at least one RNA binding domain as well as a nucleotide sequence encoding a SV40 VP1 capsid protein and/or a nucleotide sequence encoding a SV40 VP2 capsid protein.

In one another preferred embodiment, the dis-assembling in step (ii) is achieved by chemical means. In one more preferred embodiment, the chemical means are selected from the group consisting of treatment with reducing agents, preferably dithiothreitol (DTT), N-acetylcysteine (NALC), beta-mercaptoethanol, Tris(2-carboxyethyl) phosphine (TCEP), or thioredoxin.

In one example, a virus like particle (VLP) of a SV40 virus which comprises a polypeptide comprising a capsid protein of a SV40 virus or a fragment or a derivative thereof having capsid protein function and at least one RNA binding domain and an artificial circular RNA molecule comprising an RNA element of interest and a recruiting RNA motif is produced. Therefore, the virus like particle (VLP) of a SV40 virus which comprises a polypeptide comprising a capsid protein of a SV40 virus or a fragment or a derivative thereof having capsid protein function and at least one RNA binding domain is dis-assembled with dithiothreitol (DTT) at a temperature of between 35°C and 40°C, e.g. at 37°C, for a time period of between 15 and 30 minutes, e.g. 20 minutes. Specifically, a solution comprising between 10 mM and 20 mM, e.g. 15 mM, dithiothreitol (DTT) can be used. A solution comprising the artificial circular RNA molecule comprising the RNA element of interest and the recruiting RNA motif is then added to the dis-assembled virus like particle (VLP) of a SV40 virus which comprises a polypeptide comprising a capsid protein of a SV40 virus or a fragment or a derivative thereof having capsid protein function and at least one RNA binding domain. A time period of between 30 minutes and 90 minutes, e.g. 60 minutes, at a temperature of between 35°C and 40°C, e.g. at 37°C, follows. During this incubation period, the dis-assembled virus like particle (VLP) of a SV40 virus which comprises a polypeptide comprising a capsid protein of a SV40 virus or a fragment or a derivative thereof having capsid protein function and at least one RNA binding domain is loaded with the artificial circular RNA molecule comprising the RNA element of interest and the recruiting RNA motif. In general, any buffer that allows re assembly can be used. Specifically, the following loading buffer can be used: 10 mM ATP, 20 mM Hepes-KOH buffer at pH 7.9, 80 mM KCl, 40 mM NH₄Cl, 10 mM MgCl₂, 16% Glycerol, 0.08% NP-40. Subsequently, the re-assembly is induced by adding a specific salt solution [and incubation between 8 and 12 hours, e.g. overnight, at a temperature between 2 and 10 °C, e.g. at 4 °C. In general, any buffer that allows disassembling can be used. Specifically, the following reassembly buffer can be used: 150 mM sodium acetate buffer pH 5.2, 3 mM CaCl₂, 120 mM KCl and 40 mM NH₄Cl.

Due to the re-assembling, the at least one RNA binding domain binds to the recruiting RNA motif of the artificial circular RNA molecule. In this way, the artificial circular RNA molecule

(and the RNA element of interest comprised therein) is encapsidated/encapsulated in the capsid structure of the virus like particle of SV40.

Preferably, the cell referred to in the seventh and eighth aspect of the present invention is a eukaryotic cell. More preferably, the eukaryotic cell is a vertebrate cell, an arthropod cell, a yeast cell, or a fungal cell. Even more preferably, the vertebrate cell is a mammalian cell or the arthropod cell is an insect cell.

In a ninth aspect, the present invention relates to a virus like particle (VLP) obtainable by the method according to the seventh or eighth aspect. The virus like particle obtained is preferably a virus like particle according to the fifth or sixth aspect of the present invention.

In a tenth aspect, the present invention relates to a composition comprising the virus like particle according to the fifth, sixth, or ninth aspect.

Preferably, the composition is a pharmaceutical composition.

The pharmaceutical composition can be administered systemically, e.g. parenterally. For example, the pharmaceutical composition can be in a form suitable for oral administration, nasal administration, or administration by inhalation. The pharmaceutical composition can also be administered intravascular, intravenous, intramuscular, intrathecal, subcutaneous, or intraperitoneal.

The pharmaceutical composition can be administered in a single dose or in more than one dose. It is preferred that the pharmaceutical composition is to be administered in a therapeutically effective amount.

More preferably, the pharmaceutical composition comprises one or more pharmaceutically acceptable excipient(s), diluent(s), and/or carrier(s).

In this respect, it should be noted that the active component of the pharmaceutical composition is usually not the VLP itself, but the RNA element of interest comprised therein/being part of it.

In an eleventh aspect, the present invention relates to a virus like particle (VLP) according to the fifth, sixth, or ninth aspect or to a composition according to the tenth aspect for use in medicine.

In this respect, it should be noted that the active component is usually not the VLP itself, but the RNA element of interest comprised therein/being part of it.

As mentioned above, a problem of RNA based therapies so far is the delivery of biological active RNA molecules into the right cell and, thus, into the right organ at sufficiently high efficacies. Due to the fact that RNases are highly efficient in degrading RNA molecules in rather short time, long lasting effects are rather difficult to achieve and require recurring

applications of the RNA based therapy. Delivery mechanism which prevent the degradation of biological active RNA molecules would be highly desirable.

The present inventors have encapsidated/encapsulated an RNA element of interest in a virus like particle (VLP), as described herein, in particular in the capsid protein structure of a virus like particle (VLP), as described herein. In this way, the RNA element of interest is safety packaged and can efficiently be delivered into target cells, e.g. via viral routes. Within the cell/subject, the RNA element can then carry out its intended function. Due to the use of an artificial circular RNA molecule which comprises the RNA element of interest, the RNA element of interest is specifically protected against exonuclease-mediated degradation.

The RNA element of interest may be a coding or a non-coding RNA element. The RNA element of interest is preferably selected from the group consisting of a shRNA, miRNA, protein encoding RNA, pre-miRNA, piRNA, RNA complementary to other non-coding RNA, and a ribozyme. Thus, the RNA element of interest may encode, in form of a protein encoding RNA, a product of interest. A product of interest may be a protein of interest. The RNA of interest may also be a non-coding RNA, e.g. a miRNA. Non-coding RNAs are frequently used to silence a corresponding target gene.

In a twelfth aspect, the present invention relates to a virus like particle according to the fifth, sixth, or ninth aspect or to a composition according to the tenth aspect for use in therapy.

In this respect, it should be noted that the active component is usually not the VLP itself, but the RNA element of interest comprised therein/being part of it.

The present inventors have encapsidated/encapsulated an RNA element of interest in a virus like particle (VLP), as described herein, in particular in the capsid protein structure of a virus like particle (VLP), as described herein. In this way, the RNA element of interest is safety packaged and can efficiently be delivered into target cells, e.g. via viral routes. Within the cell/subject, the RNA element can then carry out its intended function. Due to the use of an artificial circular RNA molecule which comprises the RNA element of interest, the RNA element of interest is specifically protected against exonuclease-mediated degradation.

The RNA element of interest may be a coding or a non-coding RNA element. The RNA element of interest is preferably selected from the group consisting of a shRNA, miRNA, protein encoding RNA, pre-miRNA, piRNA, RNA complementary to other non-coding RNA, and a ribozyme. Thus, the RNA element of interest may encode, in form of a protein encoding RNA, a product of interest. A product of interest may be a protein of interest. The RNA of interest may also be a non-coding RNA, e.g. a miRNA. Non-coding RNAs are frequently used to silence a corresponding target gene.

Preferably, the therapy is selected from the group consisting of gene therapy, cell therapy, cancer therapy, and the treatment of a disease such as an infectious disease, e.g. caused by bacteria or viruses such as SARS-CoV-2.

More preferably, cancer therapy includes one or more of the following: (i) tumor growth inhibition and/or tumor cell death, (ii) reduction of tumor marker(s), (iii) reduction of tumor lesions and metastases, (iv) reduction of tumor burden as evidenced by imaging studies (e.g. CT, MRI, PET etc.), and (v) reduction of tumor burden as evidenced by clinical appraisal or self-report by the subject. The cancer particularly includes lung cancer, colorectal cancer, head and neck cancer, stomach cancer, urothelial cancer, breast cancer, cervical cancer, endometrial cancer, ovarian cancer, melanoma (skin cancer), pancreatic cancer, brain cancer, prostate cancer, thyroid cancer, renal cancer, adrenal cancer, liver cancer, lymphoma (cancer of the lymphocytes), or leukaemia (blood cancer).

The twelfth aspect of the present invention can alternatively be worded as follows:

A method for treating a subject comprising the step of: administering the virus like particle according to the fifth, sixth, or ninth aspect or the composition according to the tenth aspect to a subject (in need thereof), thereby treating the subject.

Preferably, the treatment is selected from the group consisting of gene therapy, cell therapy, cancer therapy, and the treatment of a disease such as an infectious disease, e.g. caused by bacteria or viruses such as SARS-CoV-2.

In a thirteenth aspect, the present invention relates to a virus like particle according to the fifth, sixth, or ninth aspect or to a composition according to the tenth aspect for use in vaccination.

In this respect, it should be noted that the active component is usually not the VLP itself, but the RNA element of interest comprised therein/being part of it.

The present inventors have encapsidated/encapsulated an RNA element of interest in a virus like particle (VLP), as described herein, in particular in the capsid protein structure of a virus like particle (VLP), as described herein. In this way, the RNA element of interest is safely packaged and can efficiently be delivered into target cells, e.g. via viral routes. Within the cell/subject, the RNA element can then carry out its intended function. Due to the use of an artificial circular RNA molecule which comprises the RNA element of interest, the RNA element of interest is specifically protected against exonuclease-mediated degradation.

The RNA element of interest may be a coding or a non-coding RNA element. The RNA element of interest is preferably selected from the group consisting of a shRNA, miRNA, protein encoding RNA, pre-miRNA, piRNA, RNA complementary to other non-coding RNA, and a

ribozyme. Thus, the RNA element of interest may encode, in form of a protein encoding RNA, a product of interest. A product of interest may be a protein of interest. The RNA of interest may also be a non-coding RNA, e.g. a miRNA. Non-coding RNAs are frequently used to silence a corresponding target gene.

The thirteenth aspect of the present invention can alternatively be worded as follows: A method for vaccinating a subject comprising the step of: administering the virus like particle according to the fifth, sixth, or ninth aspect or the composition according to the tenth aspect to a subject (in need thereof), thereby vaccinating the subject.

In one example, an RNA element encoding the spike protein of SARS-CoV-2 or parts thereof encapsidated/encapsulated into the virus like particle (VLP), as described herein, is administered to the subject for SARS-CoV-2 virus vaccination. The expression of the spike protein of SARS-CoV-2 or parts thereof is induced within the cell/subject in order to elicit an immune response against the SARS-CoV-2 virus.

In a further aspect, the present invention relates to the use of the virus like particle (VLP) according to the fifth, sixth, or tenth aspect of the present invention for artificial circular RNA molecule delivery, e.g. into the cell/subject. The artificial circular RNA molecule particularly comprises an RNA element and a recruiting RNA motif.

Alternatively formulated, the present invention relates, in a further aspect, to a method for artificial circular RNA molecule delivery, wherein the method comprises the step of: introducing the virus like particle (VLP) according to the fifth, sixth, or tenth aspect of the present invention into the cell/subject or administering the virus like particle (VLP) according to the fifth, sixth, or tenth aspect of the present invention to a subject in need thereof. The artificial circular RNA molecule particularly comprises an RNA element and a recruiting RNA motif.

It has been shown that fusion of the RNA recruiting motif to SV40 VP3 is preferred. Thus, this kind of combination is preferred in all aspects of the present invention as described herein.

If in the context of the present invention polynucleotides are defined by their DNA nucleotide sequence, polynucleotides defined by their RNA nucleotide sequence are comprised as well and *vice versa*.

Various modifications and variations of the invention will be apparent to those skilled in the art without departing from the scope of invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the

invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious to those skilled in the art in the relevant fields are intended to be covered by the present invention.

BRIEF DESCRIPTION OF THE FIGURES

The following Figures are merely illustrative of the present invention and should not be construed to limit the scope of the invention as indicated by the appended claims in any way.

FIGURE 1: Schematic presentation of an exemplarily VLP packaged with circRNA. On the left site Lentivirus was used as an example: The circRNA is fused to an Aptamer which is linked to part of one or both of the nucleocapsid (NC) or the matrix protein (MA) via an Aptamer binding protein (ABP) fused to NC and/or MA. The packaging plasmid does not encode a functional integrase protein. On the right site SV40 was used as an example: For recruitment, the circRNA is fused to an Aptamer which is linked to part of the internal capsid protein VP3 via an Aptamer binding protein (ABP) fused to VP3. VP3 binds to the internal surface of the major capsid protein VP1.

FIGURE 2: Synthesized constructs. The capsid expression constructs and fusion of capsid protein and an aptamer binding protein used in this work and a cassette that encodes the circRNA molecules to be packaged are shown. For the generation of lentiviruses VLPs a 3rd generation lentiviral system was used. Packaging and envelope plasmids encode components of the viral capsid and envelope were used, whereby a modified envelope containing aptamer binding protein (ABP) fused to NC and/or MA were used. A third plasmid that encodes the circRNA was used. For this plasmid intronic sequences from the human ZKSCAN1 genes to generate circRNAs were used. They generated circRNA consisted of the following: Strong IRES - short reporter gene (e.g. GFP) – stop and an aptamer. The highlight of the circularization is that the aptamer only becomes functional through the circularization, as it is completed through the circularization. In this way, only circRNA is packaged.

FIGURE 3: Preferred combinations of a capsid protein or a fragment or a derivative thereof fused with an RNA binding domain.

FIGURE 4: Schematic presentation of a method to load SV40 particles with RNA *in vitro*.

FIGURE 5: Real-Time PCR strategy to analyze and discriminate between linear and circular RNA. (A) PCR design that allows distinguishing between linear and circular RNA (B) PCR Product from circGFPr + circGFPf with splitCOM GFP as template. The aptamer is functional after circularization.

FIGURE 6: ddPCR based detection of linear and circular RNA within SV40 derived VLPs. SV40 derived particles were generated as described in EXAMPLE 6 either in the presence of (A) 'CircRNA COM' or (B) 'CircRNA splitCOM'. The absolute levels of linear and circular RNAs were quantified by ddPCR using the EvaGreen Digital PCR Supermix.

EXAMPLES

The examples given below are for illustrative purposes only and do not limit the invention described above in any way.

EXAMPLE 1

Design and synthesis of the synthetic nucleic acid molecules

The amino acid sequences of LV GAG (SEQ ID NO: 31), LV VSVG (SEQ ID NO: 28), SV40 VP1 (SEQ ID NO: 1), SV40 VP2 (SEQ ID NO: 2), SV40 VP2 woDBD (SEQ ID NO: 3), SV40 VP3 (SEQ ID NO: 4), SfMu Com binding protein (SEQ ID NO: 14), and a peptide linkers (GGGSGGGSGGGS; SEQ ID NO: 5) were reverse translated and nucleotide sequences were optimized by knockout of cryptic splice sites and RNA destabilizing sequence elements, optimized for increased RNA stability and adapted to match the requirements of HEK293 cells (*Homo sapiens*) regarding the codon usage. The nucleotide sequences were synthesized by GeneArt Gene Synthesis (Life technologies).

Two nucleotide constructs encoding a circRNA encoding GFP-HiBit (SEQ ID NO: 37 + 39) via protein-assisted backsplicing achieved by ZKSCAN1 introns (SEQ ID NO: 34 + 35) and comprising a SfMu Com stem loop (SEQ ID NO: 13) were designed and synthesized by GeneArt Gene Synthesis (Life technologies). In one of the constructs (SEQ ID NO: 38) the SfMu Com stem loop (SEQ ID NO: 13) was spitted and in a way that the com stem loop was only functional after circularization of the RNA. In the other construct (SEQ ID NO: 36) the SfMu Com stem loop (SEQ ID NO: 13) was functional independent of circularization of the RNA.

EXAMPLE 2

Construction of the expression plasmids

The synthesized constructs were used to generate the constructs shown in **Figure 2a**, **Figure 2b** and **Figure 2c** using standard cloning procedures. The nucleotide sequences of the generated constructs are listed here under SEQ ID NO: 17 (SV40 VP2-COM), SEQ ID NO: 19 (SV40 VP2 woDBD-COM), SEQ ID NO: 23 (SV40 COM-VP1), SEQ ID NO: 21 (SV40 VP3-COM), SEQ ID NO: 25 (SV40 VP1), SEQ ID NO: 32 (LV GAG-COM), SEQ ID NO: 27 (LV VSVG), SEQ ID NO: 29 (LV POL), SEQ ID NO: 38 (circRNA encoding GFP comprising splitCom), and SEQ ID NO: 36 (circRNA encoding GFP comprising Com). The sequences are coding for SV40 VP2-COM (SEQ ID NO: 18), SV40 VP2 woDBD-COM (SEQ ID NO: 20), SV40 COM-VP1 (SEQ ID NO: 24), SV40 VP3-COM (SEQ ID NO: 22), SV40 VP1 (SEQ ID NO: 26), LV GAG-COM (SEQ ID NO: 33), LV VSVG (SEQ ID NO: 28), LV POL (SEQ ID NO: 30), circRNA encoding GFP comprising splitCom, and circRNA encoding GFP comprising Com. The constructs were ligated into an expression vector, which allows transient transcription of the corresponding mRNA under control of the CMV promoter, terminated with a polyadenylation signal. Here, the well-known signal of the SV40 virus was used. General procedures for constructing expression plasmids are described in Sambrook, J., E.F. Fritsch and T. Maniatis: Cloning I/II/III, A Laboratory Manual New York/Cold Spring Harbor Laboratory Press, 1989, Second Edition.

EXAMPLE 3***Generation of lentivirus derived particles loaded with circular RNA by transient transfection of HEK 293 cells***

The constructs shown in **Figure 2a** were transfected into HEK 293 cells using the Effectene Transfection Reagent (Qiagen) according to the manufacturer's recommendations. In more detail, in two different experiments the following constructs were transfected at the indicated ratio: Experiment #1: 'LV-g/p COM' (SEQ ID NO: 29 and SEQ ID NO: 32), 'VSVG' (SEQ ID NO: 27), 'CircRNA COM' (SEQ ID NO: 36) (1:1:1). Experiment #2: 'LV-g/p COM' (SEQ ID NO: 29 and SEQ ID NO: 32), 'VSVG' (SEQ ID NO: 27), 'CircRNA splitCOM' (SEQ ID NO: 38) (1:1:1). Lentivirus derived particles were harvested by removing the supernatant 48 hours post transfection, followed by centrifugation (1,000 x g for 10 minutes at room temperature) and filtration through a filter with 0.45 µm pore size. The lentiviral particles were then incubated together with target cells (e.g. HEK 293 cells) for 24 hours using standard cell culturing conditions and the GFP expression was monitored by flow cytometry or fluorescence microscopy.

EXAMPLE 4

Insect cell-based generation of SV40 derived particles loaded with circular RNA by encapsidation in vitro

Based on the constructs shown in **Figure 2b** baculoviruses were generated using the Bac-to-Bac™ Baculovirus Expression System (Gibco) and the ExpiSf™ Expression System (Gibco) kits according to the manufacturer's recommendations. Briefly, the different constructs were cloned into the pFastBac donor plasmid that were then transformed into the E. coli host strain DH10Bac™ that contains a bacmid and a helper plasmid and allows generation of a recombinant bacmid following transposition of the pFastBac™ expression construct. Resulting bacmids were then isolated and transfected into Sf9 cells (derived from the fall armyworm *Spodoptera frugiperda*) using the ExpiFectamine™ Sf Transfection Reagent for fast and efficient transfection of insect cells to generate recombinant baculovirus. These recombinant baculoviruses were subsequently titrated according to the manufacturer's recommendations and stored at -80°C until further usage. To induce expression of the selected transgenes in insect cells the recombinant baculoviruses encoding the constructs shown in **Fig. 2b** were used for transduction of naïve Sf9 cells in different combinations. Therefore, defined amounts of baculoviruses, indicated as multiplicity of infection (MOI) were used for infection. The following experiments were conducted: Experiment #1: Infection with the baculovirus encoding 'SV40 VP1' (SEQ ID NO: 25) at MOI 5 combined with the infection of baculovirus encoding 'SV40 VP2 COM' (SEQ ID NO: 17) at MOI 5. Experiment #2: Infection with the baculovirus encoding 'SV40 VP1' (SEQ ID NO: 25) at MOI 5 combined with the infection of baculovirus encoding 'SV40 VP2 woDBD COM' (SEQ ID NO: 19) at MOI 5. Experiment #3: Infection with the baculovirus encoding 'SV40 VP1' (SEQ ID NO: 25) at MOI 5 combined with the infection of baculovirus encoding 'SV40 VP3 COM' (SEQ ID NO: 21) at MOI 5. Experiment #4: Infection with the baculovirus encoding 'SV40 VP1' (SEQ ID NO: 25) at MOI 5 combined with the infection of baculovirus encoding 'SV40 COM VP1' (SEQ ID NO: 23) at MOI 5. SV40 derived particles were harvested by lysing the baculovirus infected Sf9 cells 48 hours post transfection by 3x freeze/thaw steps. Cell debris was subsequently removed by centrifugation (3,200 x g for 10 minutes at room temperature) and the SV40 particles were then purified by ultracentrifugation using a 20% and 40% OptiPrep™ gradient (Abbott). The slightly diffuse band at the interphase between the 20% and 40% OptiPrep™ concentration was harvested and resulting particles were stored at -80°C or used directly for the encapsidation of the circular RNA with com sequence in vitro. Therefore, a circRNA construct containing COM

was applied as template for in vitro transcription using the Hi-T7 RNA Polymerase Kit according to manufacturer's recommendations and the initial linear RNA was subsequently circularized in vitro by adding T4 RNA ligase 2. The 'disassembly and loading process' illustrated in Fig. 4 was initiated by treating the SVV particles obtained in the experiments #1, #2, #3 and #4 with DTT (15 mM) at 37°C for 20 minutes that leads to the disassembly of the SV40 VLPs. The circular, GFP encoding 'CircRNA COM' diluted in the loading buffer (10 mM ATP, 20 mM Hepes-KOH buffer at pH 7.9, 80 mM KCl, 40 mM NH₄Cl, 10 mM MgCl₂, 16% Glycerol, 0.08% NP-40) was then added to the disassembled SV40 proteins and incubated additional 60 minutes at 37°C. Reassembly takes place by adding the reassembly buffer (150 mM sodium acetate buffer pH 5.2, 3 mM CaCl₂, 120 mM KCl and 40 mM NH₄Cl) followed by incubation at 4°C overnight. The mixtures were then treated with Salt Active Nuclease (SAN, ArcticZymes) and kept at room temperature for 10 minutes. To stop the reaction chloroform was added, and the assembled particles were recovered in the aqueous layer after centrifugation. Resulting particles were stored at -80°C or used directly for transduction of target cells. Therefore, isolated SV40 derived particles were incubated together with target cells (e.g. HEK 293 cells) for 24 hours using standard cell culturing conditions and the GFP expression was monitored by flow cytometry or fluorescence microscopy.

EXAMPLE 5

Generation of SV40 derived particles loaded with circular RNA by using a baculovirus / insect cell-based expression system

Based on the constructs shown in **Figure 2b** and **2c** baculoviruses were generated using the Bac-to-Bac™ Baculovirus Expression System (Gibco) and the ExpiSf™ Expression System (Gibco) kits according to the manufacturer's recommendations. Briefly, the different constructs were cloned into the pFastBac donor plasmid that were then transformed into the E. coli host strain DH10Bac™ that contains a bacmid and a helper plasmid and allows generation of a recombinant bacmid following transposition of the pFastBac™ expression construct. Resulting bacmids were then isolated and transfected into Sf9 cells (derived from the fall armyworm *Spodoptera frugiperda*) using the ExpiFectamine™ Sf Transfection Reagent for fast and efficient transfection of insect cells to generate recombinant baculovirus. These recombinant baculoviruses were subsequently titrated according to the manufacturer's recommendations and stored at -80°C until further usage. To induce expression of the selected transgenes in insect cells the recombinant baculoviruses encoding the constructs shown in **Fig. 2a** and **2c** were used for transduction of naïve Sf9 cells in different combinations. Therefore, defined amounts of

baculoviruses, indicated as multiplicity of infection (MOI) were used for infection. The following experiments were conducted: Experiment #1: infection with the baculovirus encoding 'SV40 VP1' (SEQ ID NO: 25) at MOI 5 combined with the infection of baculovirus encoding 'SV40 VP2 COM' (SEQ ID NO: 17) at MOI 5 plus the baculovirus 'CircRNA COM' (SEQ ID NO: 36) at MOI 5. Experiment #2: infection with the baculovirus encoding 'SV40 VP1' (SEQ ID NO: 25) at MOI 5 combined with the infection of baculovirus encoding 'SV40 VP2 woDBD COM' (SEQ ID NO: 19) at MOI 5 and 'CircRNA COM' (SEQ ID NO: 36) at MOI 5. Experiment #3: infection with the baculovirus encoding 'SV40 VP1' (SEQ ID NO: 25) at MOI 5 combined with the infection of baculovirus encoding 'SV40 VP3 COM' (SEQ ID NO: 21) at MOI 5 and 'CircRNA COM' (SEQ ID NO: 36) at MOI 5. Experiment #4: infection with the baculovirus encoding 'SV40 VP1' (SEQ ID NO: 25) at MOI 5 combined with the infection of baculovirus encoding 'SV40 COM VP1' (SEQ ID NO: 235) at MOI 5 and 'CircRNA COM' (SEQ ID NO: 36) at MOI 5. Experiment #5: infection with the baculovirus encoding 'SV40 VP1' at MOI 5 combined with the infection of baculovirus encoding 'SV40 VP2 COM' (SEQ ID NO: 17) at MOI 5 and 'CircRNA splitCOM' (SEQ ID NO: 38) at MOI 5. Experiment #6: infection with the baculovirus encoding 'SV40 VP1' (SEQ ID NO: 25) at MOI 5 combined with the infection of baculovirus encoding 'SV40 VP2 woDBD COM' (SEQ ID NO: 19) at MOI 5 and 'CircRNA splitCOM' (SEQ ID NO: 38) at MOI 5. Experiment #7: infection with the baculovirus encoding 'SV40 VP1' (SEQ ID NO: 25) at MOI 5 combined with the infection of baculovirus encoding 'SV40 VP3 COM' (SEQ ID NO: 21) at MOI 5 and 'CircRNA splitCOM' (SEQ ID NO: 38) at MOI 5. Experiment #8: infection with the baculovirus encoding 'SV40 VP1' (SEQ ID NO: 25) at MOI 5 combined with the infection of baculovirus encoding 'SV40 COM VP1' (SEQ ID NO: 23) at MOI 5 and 'CircRNA splitCOM' (SEQ ID NO: 38) at MOI 5. SV40 derived particles were harvested by lysing the baculovirus infected Sf9 cells 48 hours post transfection by 3x freeze/thaw steps. Cell debris was subsequently removed by centrifugation (3,200 x g for 10 minutes at room temperature) and the SV40 particles were then purified by ultracentrifugation using a 20% and 40% OptiPrep™ gradient (Abbott). The slightly diffuse band at the interphase between the 20% and 40% OptiPrep™ concentration was harvested and resulting particles were stored at -80°C or used directly for transduction of target cells. Therefore, isolated SV40 derived particles were incubated together with target cells (e.g. HEK 293 cells) for 24 hours using standard cell culturing conditions and the GFP expression was monitored by flow cytometry or fluorescence microscopy.

EXAMPLE 6

Generation of SV40 derived particles loaded with circular RNA by using a HEK 293 cell-based expression system

The constructs shown in **Figure 2b** and **2c** (driven by a CMV promoter) were transfected into HEK 293 cells using the Effectene Transfection Reagent (Qiagen) according to the manufacturer's recommendations. Therefore, different compositions of transfection mixes were used for the generation of SV40 derived particles containing therapeutically active RNA. In more detail, within the following experiments the specified constructs were transfected in the indicated ratio: Experiment #1: 'SV40 VP1' (SEQ ID NO: 25), 'SV40 VP2 COM' (SEQ ID NO: 17), 'CircRNA COM' (SEQ ID NO: 36) (1:1:1). Experiment #2: 'SV40 VP1' (SEQ ID NO: 25), 'SV40 VP2 woDBD COM' (SEQ ID NO: 19), 'CircRNA COM' (SEQ ID NO: 36) (1:1:1). Experiment #3: 'SV40 VP1' (SEQ ID NO: 25), 'SV40 VP3 COM' (SEQ ID NO: 21), 'CircRNA COM' (SEQ ID NO: 36) (1:1:1). Experiment #4: 'SV40 VP1' (SEQ ID NO: 25), 'SV40 COM VP1' (SEQ ID NO: 23), 'CircRNA COM' (SEQ ID NO: 36) (1:1:1). Experiment #5: 'SV40 VP1' (SEQ ID NO: 25), 'SV40 VP2 COM' (SEQ ID NO: 17), 'CircRNA splitCOM' (SEQ ID NO: 38) (1:1:1). Experiment #6: 'SV40 VP1' (SEQ ID NO: 25), 'SV40 VP2 woDBD COM' (SEQ ID NO: 19), 'CircRNA splitCOM' (SEQ ID NO: 38) (1:1:1). Experiment #7: 'SV40 VP1' (SEQ ID NO: 25), 'SV40 VP3 COM' (SEQ ID NO: 21), 'CircRNA splitCOM' (SEQ ID NO: 38) (1:1:1). Experiment #8: 'SV40 VP1' (SEQ ID NO: 25), 'SV40 COM VP1' (SEQ ID NO: 23), 'CircRNA splitCOM' (SEQ ID NO: 38) (1:1:1). SV40 derived particles were harvested by lysing the transfected HEK 293 cells 48 hours post transfection by 3x freeze/thaw steps. Cell debris was subsequently removed by centrifugation (3,200 x g for 10 minutes at room temperature) and the SV40 particles were then purified by ultracentrifugation using a 20% and 40% OptiPrep™ gradient (Abbott). The slightly diffuse band at the interphase between the 20% and 40% OptiPrep™ concentration was harvested and resulting particles were stored at -80°C or used directly for transduction of target cells. Therefore, isolated SV40 derived particles were incubated together with target cells (e.g. HEK 293 cells) for 24 hours using standard cell culturing conditions and the GFP expression was monitored by flow cytometry or fluorescence microscopy.

EXAMPLE 7

CircRNA Analytic

To measure the ration of circular RNA and linear RNA the primers circGFP_r (GAAGTTCTGCGACTGCACAC; SEQ ID NO: 41) and circGFP_f (GCATGGACGAGCTGTACAAG; SEQ ID NO: 40) or rather circGFP_r

(GAAGTTCTGCGACTGCACAC; SEQ ID NO: 41) and circIntronf (GTGTTAGCAGCACAGATCAC; (SEQ ID NO: 42) were used to amplify a small fragment within the circRNA (176 bp fragment, specific for circular RNA) or rather a fragment comprising circ5Intron (194 bp fragment, specific for linear RNA) (**Figure 5**). The following PCR conditions were applied: 95 °C for 10 min, 95 °C for 15 sec, 60 °C for 60 sec, 40 cycles. For quantification of absolute copy numbers of linear and circular RNA identical primer pairs were used in EvaGreen Digital PCR Supermix (BIO-RAD) reaction according to the manufacturer's recommendations.

Sequences of the sequence listing:

SEQ ID NO: 1	SV40 VP1	AS
SEQ ID NO: 2	SV40 VP2	AS
SEQ ID NO: 3	SV40 VP 2 woDBD	AS
SEQ ID NO: 4	SV40 VP3	AS
SEQ ID NO: 5	Linker	AS
SEQ ID NO: 6	MS2 RNA	DNA
SEQ ID NO: 7	MS2 ABP	AS
SEQ ID NO: 8	KU RNA	DNA
SEQ ID NO: 9	KU ABP1	AS
SEQ ID NO: 10	KU ABP2	AS
SEQ ID NO: 11	Sm7 RNA	DNA
SEQ ID NO: 12	SMm7 ABP	AS
SEQ ID NO: 13	COM RNA	DNA
SEQ ID NO: 14	COM ABP	AS
SEQ ID NO: 15	PP7 RNA	DNA
SEQ ID NO: 16	PP7 ABP	AS
SEQ ID NO: 17	SV40 VP2-COM	DNA
SEQ ID NO: 18	SV40 VP2-COM	AS
SEQ ID NO: 19	SV40 VP2 woDBD-COM	DNA
SEQ ID NO: 20	SV40 VP2 woDBD-COM	AS
SEQ ID NO: 21	SV40 VP3-COM	DNA
SEQ ID NO: 22	SV40 VP3-COM	AS
SEQ ID NO: 23	SV40 COM-VP1	DNA
SEQ ID NO: 24	SV40 COM-VP1	AS

SEQ ID NO: 25	SV40 VP1	DNA
SEQ ID NO: 26	SV40 VP1	AS
SEQ ID NO: 27	VSVG	DNA
SEQ ID NO: 28	VSVG	AS
SEQ ID NO: 29	POL	DNA
SEQ ID NO: 30	POL	AS
SEQ ID NO: 31	GAG	AS
SEQ ID NO: 32	GAG COM	DNA
SEQ ID NO: 33	GAG COM	AS
SEQ ID NO: 34	Circ5intron	DNA
SEQ ID NO: 35	circ3intron	DNA
SEQ ID NO: 36	CircRNA COM	DNA
SEQ ID NO: 37	GFP-HIBIT	AS
SEQ ID NO: 38	CircRNA splitCOM	DNA
SEQ ID NO: 39	GFP-HIBIT	AS
SEQ ID NO: 40	circGFPf	DNA
SEQ ID NO: 41	CircGFPr	DNA
SEQ ID NO: 42	circintronf	DNA
SEQ ID NO: 43	td circ5intron	DNA
SEQ ID NO: 44	td circ3intron	DNA
SEQ ID NO: 45 to SEQ ID NO: 185 see above		

woDBD = without DNA binding domain

AS = amino acid

If polynucleotides are defined above by their DNA nucleotide sequence, polynucleotides defined by their RNA nucleotide sequence are comprised as well and *vice versa*.

CLAIMS

1. A (linear) polynucleotide (capable of circularization) comprising in the following order from 5' to 3':
 - a first backsplicing intron sequence,
 - a first part of a recruiting RNA motif,
 - a nucleotide sequence of interest,
 - a second part of a recruiting RNA motif, and
 - a second backsplicing intron sequence,wherein the first part of the recruiting RNA motif and the second part of the recruiting RNA motif form a functional recruiting RNA motif after circularization.
2. The polynucleotide of claim 1, wherein the polynucleotide comprises between the first part of a recruiting RNA motif and the nucleotide sequence of interest an internal ribosomal entry site (IRES) or a N6-methyladenosine (m6a) for translation initiation.
3. The polynucleotide of claims 1 or 2, wherein the polynucleotide comprises at the 5' end a promoter and/or at the 3' end a poly A tail.
4. The polynucleotide of any one of claims 1 to 3, wherein the backsplicing introns are ZKSCAN1 introns.
5. The polynucleotide of any one of claims 1 to 4, wherein the circularization of the (linear) polynucleotide to a circular polynucleotide is achievable via protein-assisted backsplicing or autocatalytic backsplicing.
6. The polynucleotide of any one of claims 1 to 5, wherein the polynucleotide does not contain an in frame stop codon to enable rolling circle translation after circularization.
7. The polynucleotide of any one of claims 1 to 6, wherein the recruiting RNA motif is selected from the group consisting of a MS2 phage operator stem-loop, a telomerase Ku binding motif, a telomerase Sm7 binding motif, a SfMu phage COM stem-loop, and a PP7 phage operator stem-loop.

8. The polynucleotide of any one of claims 1 to 7, wherein the polynucleotide is a DNA or an RNA polynucleotide.
9. A vector comprising the polynucleotide of any one of claims 1 to 8.
10. An artificial circular RNA molecule comprising an RNA element of interest and a recruiting RNA motif.
11. The artificial circular RNA molecule of claim 10, wherein the recruiting RNA motif is selected from the group consisting of a MS2 phage operator stem-loop, a telomerase Ku binding motif, a telomerase Sm7 binding motif, a SfMu phage COM stem-loop, and a PP7 phage operator stem-loop.
12. The artificial circular RNA molecule of claim 11, wherein the recruiting RNA motif has a nucleotide sequence selected from the group consisting of SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15 (or is a nucleotide sequence transcribed thereof), and a nucleotide sequence having at least 80% sequence identity thereto.
13. The artificial circular RNA molecule of any one of claims 10 to 12, wherein the RNA element of interest is selected from the group consisting of a shRNA, miRNA, protein coding RNA, pre-miRNA, piRNA, RNA complementary to other non-coding RNA, and a ribozyme.
14. A complex of
 - (i) a polypeptide comprising a capsid protein of a virus or a fragment or a derivative thereof having capsid protein function and at least one RNA binding domain, and
 - (ii) an artificial circular RNA molecule comprising an RNA element of interest, preferably the artificial circular RNA molecule of any one of claims 10 to 13.
15. The complex of claim 14, wherein the artificial circular RNA molecule comprises a recruiting RNA motif.

16. The complex of claim 15, wherein the at least one RNA binding domain binds to the recruiting RNA motif.
17. The complex of any one of claims 14 to 16, wherein the at least one RNA binding domain is connected to/fused to/associated with the capsid protein of a virus or a fragment or a derivative thereof having capsid protein function, preferably via a linker.
18. The complex of claim 17, wherein the at least one RNA binding domain is connected to/fused to/associated with the N-terminus and/or C-terminus of the capsid protein of a virus or a fragment or a derivative thereof having capsid protein function, preferably via a linker.
19. The complex of any one of claims 14 to 18, wherein the at least one RNA binding domain is a naturally occurring RNA binding domain or an artificial RNA binding domain.
20. The complex of any one of claims 14 to 19, wherein the at least one RNA binding domain is selected from the group consisting of a MS2 coat protein or an RNA-binding section thereof, a Ku protein or an RNA-binding section thereof, a Sm7 protein or an RNA-binding section thereof, a SfMu phage COM RNA binding protein or an RNA-binding section thereof, and a PP7 coat protein or an RNA-binding section thereof.
21. The complex of any one of claims 15 to 20, wherein the recruiting RNA motif is selected from the group consisting of a MS2 phage operator stem-loop, a telomerase Ku binding motif, a telomerase Sm7 binding motif, a SfMu phage COM stem-loop, and a PP7 phage operator stem-loop.
22. The complex of any one of claims 14 to 21, wherein
 - (i) the at least one RNA binding domain is a MS2 coat protein or an RNA-binding section thereof and the recruiting RNA motif is a MS2 phage operator stem-loop,
 - (ii) the at least one RNA binding domain is a Ku protein or an RNA-binding section thereof and the recruiting RNA motif is a telomerase Ku binding motif,
 - (iii) the at least one RNA binding domain is a Sm7 protein or an RNA-binding section thereof and the recruiting RNA motif is a telomerase Sm7 binding motif,

- (iv) the at least one RNA binding domain is a SfMu phage COM RNA binding protein or an RNA-binding section thereof and the recruiting RNA motif is a SfMu phage COM stem-loop, and/or
 - (v) the at least one RNA binding domain is a PP7 coat protein or an RNA-binding section thereof and the recruiting RNA motif is PP7 phage operator stem-loop.
23. The complex of any one of claims 20 to 22, wherein the at least one RNA binding domain has an amino acid sequence selected from the group consisting of SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, and an amino acid sequence having at least 80% sequence identity thereto.
24. The complex of any one of claims 21 to 23, wherein the recruiting RNA motif has a nucleotide sequence selected from the group consisting of SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15 (or is a nucleotide sequence transcribed thereof), and a nucleotide sequence having at least 80% sequence identity thereto.
25. The complex of any one of claims 14 to 24, wherein the RNA element of interest is selected from the group consisting of a shRNA, miRNA, protein encoding RNA, pre-miRNA, piRNA, RNA complementary to other non-coding RNA, and a ribozyme.
26. The complex of any one of claims 14 to 25, wherein the virus is a DNA or RNA virus.
27. The complex of claim 26, wherein
the DNA virus is selected from the group consisting of a papovavirus, a papilloma virus, a polyoma virus, an adenovirus, and a parvovirus virus, or
the RNA virus is a retrovirus, a norovirus, or an orthomyxovirus.
28. The complex of claim 27, wherein
the polyoma virus is a Simian Virus 40 (SV40), a BK virus (BKV), a JC virus (JCV), or a MPy virus (MPyV),
the parvovirus is an adeno-associated virus (AAV),
the retrovirus is a lentivirus,
the norovirus is a Norwalk virus, or

the orthomyxovirus is an influenza A or influenza B virus.

29. The complex of any one of claims 14 to 28, wherein the capsid protein is a capsid protein of an RNA or a DNA virus.
30. The complex of claim 29, wherein
the capsid protein of a DNA virus is selected from the group consisting of a papovavirus capsid protein, a papilloma virus capsid protein, a polyoma virus capsid protein, an adenovirus capsid protein, and a parvovirus capsid protein, or
the capsid protein of an RNA virus is a lentivirus capsid protein.
31. The complex of claim 30, wherein
 - (i) the parvovirus capsid protein is an adeno-associated virus capsid protein, preferably an AAV9 capsid protein,
 - (ii) the polyoma virus capsid protein is a SV40 capsid protein, a BKV capsid protein, a JCV capsid protein, or a MPyV capsid protein, or
 - (iii) the lentivirus capsid protein is a nucleocapsid (NC) protein, a matrix protein (MA) or a capsid protein (CA).
32. The complex of claim 31, wherein
 - (i) the AAV9 capsid protein is an AAV9 VP1 capsid protein, an AAV9 VP2 capsid protein, or an AAV9 VP3 capsid protein,
 - (ii) the SV40 capsid protein is a SV40 VP1 capsid protein, a SV40 VP2 capsid protein, or a SV40 VP3 capsid protein,
 - (iii) the BKV capsid protein is a BKV VP1 capsid protein, a BKV VP2 capsid protein, or a BKV VP3 capsid protein,
 - (iv) the JCV capsid protein is a JCV VP1 capsid protein, a JCV VP2 capsid protein, or a JCV VP3 capsid protein, or
 - (v) the MPyV capsid protein is a MPyV VP1 capsid protein, a MPyV VP2 capsid protein, or a MPyV VP3 capsid protein.
33. The complex of claims 31 or 32, wherein
the SV40 VP1 capsid protein has an amino acid sequence according to SEQ ID NO: 1
or an amino acid sequence having at least 80% sequence identity thereto,

the SV40 VP2 capsid protein has an amino acid sequence according to SEQ ID NO: 2 or an amino acid sequence having at least 80% sequence identity thereto,
the SV40 VP2 capsid protein without DNA binding domain (woDBD) has an amino acid sequence according to SEQ ID NO: 3 or an amino acid sequence having at least 80% sequence identity thereto,
the SV40 VP3 capsid protein has an amino acid sequence according to SEQ ID NO: 4 or an amino acid sequence having at least 80% sequence identity thereto,
the nucleocapsid (NC) protein has an amino acid sequence encompassed by amino acid sequence according to SEQ ID NO: 31 or an amino acid sequence having at least 80% sequence identity thereto,
the matrix protein (MA) has an amino acid sequence encompassed by amino acid sequence according to SEQ ID NO: 31 or an amino acid sequence having at least 80% sequence identity thereto, or
the capsid protein (CA) has an amino acid sequence encompassed by amino acid sequence according to SEQ ID NO: 31 or an amino acid sequence having at least 80% sequence identity thereto.

34. A virus like particle (VLP) which comprises the complex of any one of claims 14 to 33.
35. A virus like particle (VLP) which comprises an artificial circular RNA molecule comprising an RNA element of interest.
36. The VLP of claim 35, wherein the artificial circular RNA molecule is encapsidated in the VLP.
37. The VLP of claims 35 or 36, wherein the VLP comprises the complex of any one of claims 14 to 33.
38. The VLP of any one of claims 34 to 37, wherein the VLP is a DNA or an RNA VLP.
39. The VLP of claim 38, wherein
the DNA VLP is a VLP of a virus selected from the group consisting of a papovavirus, a papilloma virus, a polyoma virus, an adenovirus, and a parvovirus virus, or
the RNA VLP is a VLP of a retrovirus, a norovirus, or an orthomyxovirus.

40. The VLP of claim 39, wherein
the polyoma virus is a Simian Virus 40 (SV40), a BK virus (BKV), a JC virus (JCV), or
a MPy virus (MPyV),
the parvovirus is an adeno-associated virus (AAV),
the retrovirus is a lentivirus,
the norovirus is a Norwalk virus, or
the orthomyxovirus is an influenza A or influenza B virus.
41. A method for producing a virus like particle (VLP) which comprises an artificial circular
RNA molecule comprising an RNA element of interest, preferably as defined in any one
of claims 34 to 40, comprising the steps of:
- (i) providing a cell, and
 - (ii) introducing
 - (iia) a polypeptide comprising a capsid protein of a virus or a fragment or a
derivative thereof having capsid protein function and at least one RNA
binding domain, a polynucleotide encoding the polypeptide, or a vector
comprising the polynucleotide, and
 - (iib) a polynucleotide according to any one of claims 1 to 8, a vector
according to claim 9, an artificial circular RNA molecule comprising
an RNA element of interest, or an artificial circular RNA molecule
according to any one of claims 10 to 13into the cell,
thereby producing the virus like particle (VLP) comprising an artificial circular
RNA molecule comprising an RNA element of interest.
42. A method for producing a virus like particle (VLP) which comprises an artificial circular
RNA molecule comprising an RNA element of interest, preferably as defined in any one
of claims 34 to 40, comprising the steps of:
- (i) providing a virus like particle (VLP) which comprises a polypeptide comprising
a capsid protein of a virus or a fragment or a derivative thereof having capsid
protein function and at least one RNA binding domain,
 - (ii) dis-assembling the VLP,

- (iii) adding an artificial circular RNA molecule comprising an RNA element of interest or an artificial circular RNA molecule according to any one of claims 10 to 13,
 - (iv) re-assembling the dis-assembled VLP,
thereby producing the virus like particle (VLP) comprising an artificial circular RNA molecule comprising an RNA element of interest.
43. The method of claim 42, wherein the virus like particle (VLP) which comprises a polypeptide comprising a capsid protein of a virus and at least one RNA binding domain provided in (i) is produced by
- (a) providing a cell, and
 - (b) introducing a polypeptide comprising a capsid protein of a virus or a fragment or a derivative thereof having capsid protein function and at least one RNA binding domain, a polynucleotide encoding the polypeptide, or a vector comprising the polynucleotide into the cell.
44. The method of claims 42 or 43, wherein the dis-assembling is achieved by chemical means.
45. The method of claim 44, wherein the chemical means are selected from the group consisting of treatment with reducing agents, preferably dithiothreitol (DTT), N-acetylcysteine (NALC), beta-mercaptoethanol, Tris(2-carboxyethyl) phosphine (TCEP), or thioredoxin.
46. The method of any one of claims 41 to 45, wherein the cell is a eukaryotic cell.
47. The method of claim 46, wherein the eukaryotic cell is a vertebrate cell, an arthropod cell, a yeast cell, or a fungal cell.
48. The method of claim 47, wherein the vertebrate cell is a mammalian cell or the arthropod cell is an insect cell.

49. The method of any one of claims 41 to 48, wherein the RNA element of interest is selected from the group consisting of a shRNA, miRNA, protein encoding RNA, pre-miRNA, piRNA, RNA complementary to other non-coding RNA, and a ribozyme.
50. A virus like particle (VLP) obtainable by the method of any one of claims 41 to 49.
51. A composition comprising the virus like particle of any one of claims 34 to 40 or 50.
52. The composition of claim 51, wherein the composition is a pharmaceutical composition.
53. The composition of claim 52, wherein the pharmaceutical composition comprises one or more pharmaceutically acceptable carriers.
54. A virus like particle of any one of claims 34 to 40 or 50 or a composition of any one of claims 51 to 53 for use in medicine.
55. A virus like particle of any one of claims 34 to 40 or 50 or a composition of any one of claims 51 to 53 for use in therapy.
56. A virus like particle of any one of claims 34 to 40 or 50 or a composition of any one of claims 51 to 53 for use in vaccination.